nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Microbiome data processing was performed using the DADA2 workflow for big datasets (v. 1.10.42, https://benjjneb.github.io/dada2/bigdata.html), resulting in abundance tables of amplicon sequence variants (ASVs).
Data analysis	The data analysis was performed in python v3.8.1 based on the packages NetworkX v2.2 (https://github.com/networkx/networkx), safepy (https://github.com/baryshnikova-lab/safepy), scikit-learn v1.5.1 (https://github.com/scikit-learn/scikit-learn) and tmap v1.2 (https://github.com/GPZ-Bioinfo/tmap) as well as R v4.4.0 based on the package vegan v2.6-6.1.55,56 Data visualization was based on plotly v5.22 (https://github.com/plotly/plotly.py) and iTOL v6 (https://itol.embl.de/). HTML versions of the presented plots can be found on OSF allowing interactive data exploration (https://osf.io/9ykv5/).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

HCHS data can be obtained by qualified researchers on reasonable request to the study's steering committee. The analysis code for this work is publicly available on GitHub (https://github.com/csi-hamburg/oral_microbiome_brain_health). Interactive versions of the plots can be found on OSF (https://osf.io/9ykv5).

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Sex was recorded via self-report (42.7% female), while gender was not separately assessed in our study. Sex was included as a covariate in statistical models to account for its potential influence on oral microbiome composition and brain health phenotypes. No informed consent has been obtained for publicly sharing individual-level study data.
Reporting on race, ethnicity, or other socially relevant groupings	In our study, no variables on race or ethnicity were used, as the vast majority of participants were of European descent, and the representation of other ethnicities was too small to allow for robust statistical analyses.
Population characteristics	The study included 1,026 participants with a mean age of 63.72 years (SD ± 8.17), of whom 42.7% were female (n = 438). Education level, assessed using the International Standard Classification of Education (ISCED), had a mean score of 2.42 (SD ± 0.58), reflecting a predominantly intermediate educational background.
Recruitment	PAROMIND is nested in the Hamburg City Health Study (HCHS). HCHS recruits participants through a random sampling of 45,000 inhabitants of Hamburg, Germany, aged 45–74 years, drawn from official population registers. Invitations were sent by mail, and participation was voluntary, requiring individuals to schedule an appointment at a dedicated study center. Potential self-selection bias may arise due to voluntary participation, as individuals with higher health awareness, greater education, or specific health concerns may be more likely to enroll. Additionally, socioeconomic and demographic differences in response rates could influence the representativeness of the cohort. These biases may affect the generalizability of findings, particularly regarding health behaviors and risk factor distributions.
Ethics oversight	PAROMIND and the HCHS were approved by the local ethics committee of the Landesärztekammer Hamburg (State of Hamburg Chamber of Medical Practitioners, PV5131). The conduct of PAROMIND is governed by ethical guidelines of Good Clinical Practice (GCP), Good Epidemiological Practice (GEP) and the Declaration of Helsinki.39 Written informed consent was obtained from all participants investigated in this work.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The sample size of 1,026 participants was determined based on the number of individuals recruited at the time of analysis from the Hamburg City Health Study (HCHS). No formal power calculations were performed; instead, all available participants meeting the study criteria were included to maximize statistical power and generalizability.
Data exclusions	Participants were excluded if their oral microbiome or neuroimaging data did not meet quality standards. Microbiome samples with fewer than 10,000 sequences were excluded, and genera with a frequency below 0.1% in a sample were discarded. Neuroimaging data quality was assessed using established procedures published elsewhere (https://www.pnas.org/doi/10.1073/pnas.2217232120).
Replication	To verify the robustness of our results against variations in the examined sample as well as the analysis pipeline, we conducted a comprehensive sensitivity analysis. This involved repeating the entire analysis across random subsamples of different sizes. Specifically, we varied the sample sizes from 100% to 10% of the total dataset, in 1% decrements. For each decrement, we randomly sampled subsets 100 times, resulting in a total of 9,000 iterations (90 different sample sizes × 100 random samples per steps). For each iteration, we assessed the robustness of our findings by

comparing the results from the subsamples to the original results. The stability of the enrichment analysis results was evaluated by calculating the Spearman correlation of enrichment ratios for the non-microbiome phenotypes and genus-level abundance. In addition to this, we measured the agreement of the participant-group assignments resulting from k-Means clustering used for group analysis employing the Adjusted Rand Index (ARI) which ranges from 0 (no agreement) to 1 (full agreement).
Our analysis pipeline involved several design choices, which are based on best practices or are the default settings of the software tools used. To ensure that our results were not biased by these design choices, we performed a sensitivity analysis with different pipeline configurations. We systematically explored variations in parameter values and pipeline components, altering the Mapper cover overlap from the original 0.75 to 0.5 and 0.99; the Mapper cover resolution from 30 to 10, 20, 40, and 50; the Mapper epsilon threshold from 0.95 to 0.99, 0.90, and 0.85; and the Mapper filter function from principal coordinate analysis of the Bray-Curtis dissimilarity to multidimensional scaling and Uniform Manifold Approximation and Projection (UMAP) on the raw data. Additionally, we adjusted the SAFE distance threshold from 0.75 to 0.5 and 0.99, and the SAFE neighborhood radius from 0.1 to 0.05 and 0.15. We adjusted one parameter from the original pipeline per iteration, resulting in a total of 15 iterations. The robustness of our results was evaluated by comparing the findings from the original configuration to those from alternative setups. Matching the approach for the previous sensitivity analysis, we calculated the Spearman correlation of enrichment ratios for clinical and genus abundance phenotypes and the ARI for group assignments from k-Means clustering to assess stability.

Randomization: Not applicable.

Blinding: Not applicable.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☐	☒ MRI-based neuroimaging

Plants

Seed stocks: *Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*

Novel plant genotypes: *Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*

Authentication: *Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

Magnetic resonance imaging

Experimental design

Design type: Structural neuroimaging; not applicable

Design specifications: Not applicable

Behavioral performance measures: Not applicable

Acquisition

Imaging type(s): Structural imaging; diffusion-weighted imaging

Field strength: 3 Tesla

Sequence & imaging parameters: For 3D T1-weighted anatomical images, rapid acquisition gradient-echo sequence (MPRAGE) was used with the following sequence parameters: TR=2500 ms, TE=2.12 ms, 256 axial slices, ST=0.94 mm, and IPR=0.83×0.83 mm.

Area of acquisition: Whole brain scan was used

Diffusion MRI ☒ Used ☐ Not used

Parameters: For single-shell diffusion-weighted imaging, 75 axial slices were obtained covering the whole brain with gradients (b=1000 s/mm2) applied along 64 noncollinear directions with the following sequence parameters: repetition time (TR)=8500 ms, echo time (TE)=75 ms, slice thickness (ST)=2 mm, in-plane resolution (IPR)=2×2 mm, anterior–posterior phase-encoding direction, 1 b0 volume.

Preprocessing

Preprocessing software: The T1-weighted image was corrected for intensity non-uniformity (INU) using N4BiasField-Correction (ANTs 2.3.1),. The T1w-reference was then skull-stripped using antsBrainExtraction.sh (ANTs 2.3.1), using OASIS as target template.

QSIPrep 0.14.21 was used for preprocessing of diffusion-weighted imaging.

Normalization: Not applicable.

Normalization template: Not applicable.

Noise and artifact removal: Noise and artifact removal were applied on diffusion-weighted imaging. MP-PCA denoising with a 5-voxel window was applied using MRtrix3, followed by Gibbs unringing and B1 field inhomogeneity correction with the N4 algorithm. Head motion and eddy current corrections were performed using FSL's eddy, which applied q-space smoothing, outlier replacement, and post-eddy shell alignment. Susceptibility distortions were corrected using fMRIPrep's fieldmap-less approach, with registration performed via ANTs, constraining deformation along the phase-encoding direction. Finally, confounding time-series, including head-motion estimates and slicewise cross-correlation, were calculated, and the DWI time-series were resampled to ACPC space at 2mm isotropic resolution.

Volume censoring: Not applicable.

Statistical modeling & inference

Model type and settings: Multivariable regression.

Effect(s) tested: Not applicable.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016): No cluster- or voxel-level statistics were performed. Instead global imaging metrics were statistically assessed.

Correction: All statistics were corrected for multiple comparisons.

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis