Supplementary Material

Enlarged Perivascular Spaces Are Independently Associated with High Pulse Wave Velocity: A Cross-Sectional Study

SUPPLEMENTARY METHODS

Outline of the Gimlet study

Subjects

Between March 2016 and March 2017, we enrolled 128 patients visiting the memory clinic at the National Center for Geriatrics and Gerontology (NCGG) in Japan who agreed to undergo a medical assessment of their cognitive function and a fecal examination. Patients were eligible for the Gimlet study if they met the following criteria: (1) were able to undergo a brain MRI; (2) provided informed consent in writing; (3) provided informed consent for the NCGG Biobank to store their clinical data, blood, and fecal samples; and (4) were accompanied by a study partner who could assess the patient's condition. Patients were excluded if they met the following criteria: (1) were unable to undergo an MRI examination or the MRI was unable to be evaluated because of movement; (2) had local lesions, such as cerebral infarctions, that were detected by MRI before enrolment and might significantly affect cognitive function; (3) had a history of a major psychological disorder or current, serious, or unstable alcohol or drug abuse; (4) had ≤6 years of education; (5) had a history of cancer of the digestive tract; or (6) were judged by an investigator to be ineligible to participate as a study subject (e.g., recent use of antibiotics, digestive disorder such as liver cirrhosis or inflammatory bowel disease, neurological disorder such as brain tumor, encephalitis/meningitis, normal pressure hydrocephalus, or Huntington's disease). In 2022, 22 participants presenting with either DLB, mild cognitive impairment (MCI),

or normal cognition (NC) were additionally enrolled to the Gimlet study to assess the relationship between DLB and gut microbiota.

Baseline assessment

All participants underwent a comprehensive geriatric assessment[1] using the following: (1) demographic characteristics; (2) risk factors, such as hypertension, dyslipidemia, diabetes mellitus, ischemic heart disease, chronic kidney disease, smoking habits, or a history of stroke and alcohol consumption; (3) basic and instrumental activities of daily living (ADL) scales, assessed using the Barthel Index[2] and Lawton and Brody scale;[3] (4) global cognitive function, assessed using the Mini-Mental State Examination (MMSE)[4] and Clinical Dementia Rating (CDR) scales;[5] (5) neuropsychological testing, using the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-cog),[6] Raven's Coloured Progressive Matrices (RCPM),[7] Frontal Assessment Battery (FAB),[8] and Logical Memory subtests I and II of the Wechsler Memory Scale-Revised (LM-WMSR);[9] (6) behavioral and psychological symptoms, assessed using the Dementia Behaviour Disturbance Scale (DBDS);[10] (7) burden of caregivers, assessed by the Zarit Caregiver Burden Interview (ZBI);[11] (8) depressive status, assessed by the Geriatric Depression Scale (GDS);[12] (9) laboratory variables, including *APOE* ε4 as a risk factor for AD; (10) ankle brachial index and pulse wave velocity, as indicators of arteriosclerosis[13] and the 'impact' of pulse;[14] (11) brain imaging, such as MRI and SPECT; (12) an assessment of other factors, such as the presence of frailty[15] and subjective hearing loss; and (13) an assessment of social and lifestyle factors, such as the Mini-Nutritional Assessment-Short Form (MNA-SF) to assess nutritional status.[16] All clinical samples and data were provided by the NCGG Biobank, which collects clinical data for research.

Risk factors

Hypertension was defined by a systolic blood pressure of ≥140 mmHg or a diastolic blood pressure of ≥90 mmHg, and/or the use of anti-hypertensive drugs. Dyslipidemia was defined by a serum low-density lipoprotein cholesterol concentration of ≥140 mg/dL, a serum high-density lipoprotein cholesterol concentration of <40 mg/dL, a serum triacylglycerol concentration of ≥150 mg/dL, and/or the use of statins. Diabetes mellitus was defined by a hemoglobin A1c concentration of ≥6.5%, and/or the use of oral hypoglycemic drugs or insulin, and/or a fasting serum glucose concentration of ≥69.9 mol/L (126 mg/dL). Ischemic heart disease was defined by a history of physician-diagnosed angina pectoris and/or evidence of a prior myocardial infarction or coronary revascularization procedure (percutaneous coronary intervention or coronary artery bypass surgery). Serum creatinine was measured and the estimated glomerular filtration rate (eGFR) was determined using the equation proposed by the Japanese Society of Nephrology, as follows: eGFR (mL/min/1.73 m^2) = 194 × (serum creatinine [mg/dL])$^{-1.094}$ × (age [years])$^{-0.287}$ (× 0.739 if female). Chronic kidney disease (CKD) was defined by an eGFR of <60 mL/min/1.73 m^2. We performed blood count and biochemical examination.

MRI imaging and SPECT imaging

The participants underwent a 1.5-T MRI of their brains (Philips Ingenia, Eindhoven, the Netherlands). MRI scans were obtained, including diffusion-weighted images, fluid-attenuated inversion recovery images, T2-weighted images, $T2^*$-weighted gradient-echo images, three-dimensional T1-weighted sagittal and axial coronal views, and 3D time-of-flight magnetic resonance angiography scans. The presence and components of cerebral small vessel disease, such as silent lacunar infarct (SLI), white matter hyperintensity (WMH), cerebral microbleeds

(CMB), and enlarged periventricular space (EPVS), were categorized using previously published standards for reporting vascular changes on neuroimaging.[17, 18] The voxel-based specific regional analysis system for Alzheimer's Disease (VSRAD) software (Eisai Co., Ltd., Tokyo, Japan) was used to quantify cortical and hippocampal atrophy, using standardized z-scores.[19] A high VSRAD score suggests the presence of Alzheimer's disease (AD) because this score reflects hippocampal atrophy, which is one of the characteristics of the brain of a patient with AD. The participants also underwent N-isopropyl-p-[^{123}I]-iodoamphetamine-SPECT, in which the presence of low blood flow in the area of the posterior cingulate gyrus and/or the precuneus was regarded as a surrogate marker of AD.[20]

MRI features of SVD

The presence and components of cerebral small vessel disease (SVD) were categorized according to the Standards for Reporting Vascular Changes on Neuroimaging recommendations.[17, 18] We defined SLI as a focal lesion of ≥3 mm in diameter that was hyperintense on T2-weighted imaging and hypointense on FLAIR images. We defined WMH as periventricular and deep white matter hyperintense lesions on T2-weighted and FLAIR images. An irregular periventricular hyperintensity (Fazekas grade ≥ 3) and/or early confluent or confluent separate deep white matter hyperintense lesions (Fazekas grade ≥ 2) were defined as severe WMH. We defined CMB as a focal area of signal loss in the brain parenchyma of <5 mm on a T2*-weighted gradient-echo imaging scan. We defined EPVS as small (<3 mm), punctate (if perpendicular to the plane of the scan) or linear (if longitudinal to the plane of the scan) hyperintensities on T2 images in the basal ganglia or centrum semiovale, as previously reported.[21] BG-EPVS and CSO-EPVS were coded with the following scale, applied to standard axial images: 0 (no EPVS); 1 (<10 EPVS); 2 (11–20 EPVS); 3 (21–40 EPVS); and 4 (>40

EPVS).[22]

Inter/intra-rater reliabilities

In this sub-analysis, Cohen's kappa coefficient was calculated by using the MRI data of a randomly selected subset of 20 participants to assess the inter-rater reliability (N.S. and Y.K.). The values of inter-rater reliabilities as follows: 1.00 for SLI, 0.78 for WMH, 0.83 for CMB, 1.00 for css, 0.76 for BG-EPVS, and 1.00 for CSO-EPVS. In addition, intra-rater reliability (N.S.) was also assessed for the quality of the assessment regarding each component of cerebral SVD. The values of intra-rater reliabilities as follows: 0.77 for SLI, 1.00 for WMH, 1.00 for CMB, 1.00 for css, 0.89 for BG-EPVS, and 1.00 for CSO-EPVS. Kappa values between 0.76 and 1.00 were interpreted as adequate agreement.[23]

Total SVD score

Based on a previous study,[24] we rated the total MRI burden of SVD on an ordinal scale from 0 to 4, by summing the presence of each of four features of cerebral SVD. This score consists of the following: (1) SLI (1 point if present); (2) CMB (1 point if present); (3) EPVS (1 point if moderate to severe EPVS are present in the basal ganglia [grades 2–4]); and (4) WMH (1 point for the presence of either [early] confluent deep WMH [Fazekas score 2 or 3] or irregular periventricular WMH extending into the deep white matter [Fazekas score 3]).

Classification of cognitive function

Clinical Dementia Rating (CDR) is a 5-point scale, which consists of six domains of cognitive and functional performance: memory, orientation, judgment and problem solving,

community affairs, home and hobbies, and personal care. After ratings for each domain, a CDR global score was calculated according to the official scoring rules.[5] The CDR global score is indicative of cognitive impairment/dementia as shown below: 0 = normal (no memory loss or slight inconsistent forgetfulness, independent function in a daily life); 0.5 = very mild dementia including mild cognitive impairment (MCI: consistent slight forgetfulness or partial recollection of events, benign forgetfulness, slight impairment in a daily life); 1 = mild dementia; 2 = moderate dementia; and 3 = severe dementia. We also assessed a CDR Sum of Boxes (CDR-SB) for a quantitative analysis. In this study, participants were categorized as having either MCI or normal cognition (NC). MCI was defined as an MMSE score ≥ 20 and a CDR global score of 0.5, which indicates possible, very mild dementia and a higher risk of developing dementia.[13] NC was defined as an MMSE score ≥ 20 and a CDR global score of 0.

Plasma neurofilament light chain

Blood samples were collected, processed onsite to isolate plasma, aliquoted, and frozen at −81°C in the NCGG Biobank. Plasma NfL concentrations were measured according to manufacturer instructions using the NF-Light Advantage Kit on a highly sensitive single-molecule array assay Simoa HD-1 platform (Quanterix, Lexington, MA, USA).[25, 26] Plasma samples were measured at a 1:4 dilution and were run in duplicate by a trained technician. All samples were measured blinded. All NfL values were within the linear ranges of the assays.

Sample collection

Patients or their family members used a fecal sampler to collect a fecal sample as soon as possible after the patient's bowel movement. The sample was placed in a specimen container. Patients collected fecal samples on the day of their hospital consultation, and the samples were

presented to the clinical laboratory center of the NCGG (preferably within 4 h of the bowel movement). Because one of the inclusion criteria for our study was that patients presented with study partners (family members), all of the demented patients were able to be supported if they needed support about excretion. Furthermore, when we received the fecal samples, we only accepted appropriate samples as per the analyzing company's criteria, while inadequate samples were disposed of and resubmission was requested. The samples were frozen and stored at −81°C in the NCGG Biobank. After all samples had been collected, they were transported (frozen) to TechnoSuruga Laboratory (Shizuoka, Japan).

Gut microbiome

Fecal samples were collected at home, just after a bowel evacuation, by patients or their family members. The samples were collected using scoop collection tubes, and patients consumed their usual diets before and after sample collection. Samples were frozen and preserved at -81°C at the NCGG Biobank. After all samples had been collected, the gut microbiomes were analyzed by TechnoSuruga Laboratory (Shizuoka, Japan) using T-RFLP analysis.[27] T-RFLP analysis is one of the most well-established and reliable 16S ribosomal RNA-based methods, especially when considering its high throughput and reproducibility. First, T-RFLP was used to classify gut microbes into the following 10 groups: *Prevotella*, *Bacteroides*, Lactobacillales, *Bifidobacterium*, *Clostridium* cluster IV, *Clostridium* subcluster XIVa, *Clostridium* cluster IX, *Clostridium* cluster XI, *Clostridium* cluster XVIII, and 'others'. Second, by referencing the Human Fecal Microbiome T-RFLP profile[28, 29], the gut microbiome was stratified into three enterotypes: enterotype I included *Bacteroides* at > 30%, enterotype II included *Prevotella* at > 15%, and enterotype III included the remaining bacteria. Third, the F/B ratio was examined, because an increase in F/B ratio indicates dysbiosis.[30] The phylum

Firmicutes included the Lactobacillales and *Clostridium* clusters, and the phylum Bacteroidetes included *Bacteroides* and *Prevotella*.

Terminal restriction fragment length polymorphisms

Fecal samples (approximately 10 mg each) were suspended in 900 μL of a solution containing 100 mM tris-HCl (pH 9.0), 40 mM ethylenediaminetetraacetic acid, 4 M guanidine thiocyanate and 0.001% bromothymol blue. Fecal solids in the suspension were broken down using a FastPrep FP100A Instrument (MP Biomedicals; CA, USA) and zirconia beads at 5 m/s for 2 min. DNA was then extracted from 200 μL of suspension using an automatic nucleic acid extractor and MagDEA® DNA 200 (Precision System Science, Chiba, Japan). PCR was performed using a Takara Thermal Cycler Dice TP650 (Takara Bio, Shiga, Japan) in a reaction mixture (20 μl) containing 1×PCR buffer, each deoxynucleoside triphosphate at a concentration of 200 μM, 1.5 mM $MgCl_2$, each primer at a concentration of 0.2 μM, 10 ng of fecal DNA and 0.2 U of HotStar Taq DNA polymerase (Qiagen, Hilden, Germany). The primers used were 5' fluorescein amidite-labeled 516f (5'-TGC- CAGCAGCCGCGGTA-3'; *Escherichia coli* positions 516–532) and 1510r (5'-GGTTACCTTGTTACGA- CTT-3'; *E. coli* positions 1510–1492). The amplification program used was as follows: preheating at 95°C for 15 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 90 s; and finally, a terminal extension at 72°C for 10 min. The size of the amplicon was verified by electrophoresis and it was purified using a MultiScreen PCR96 Filter Plate (Millipore, Billerica, MA, USA). The purified 16S rDNA amplicons were treated with 10 U of FastDigest *BseLI* (Thermo Fisher Scientific., MA, USA) for 10 min. An ABI PRISM 3130×l genetic analyzer (Thermo Fisher Scientific) was used to analyze the resultant fluorescence-labeled terminal restriction fragments (T-RFs), and GeneMapper software (Thermo Fisher Scientific) was used to

determine T-RF length and peak area for each sample. T-RFs were divided into 29 operational taxonomic units (OTUs). The OTUs were quantified as the percentage of each OTU of the total OTU area and expressed as the percentage of the area under the curve (% AUC). The reference database, Human Fecal Microbiota T-RFLP profiling (http://www.tecsrg-lab.jp/), was used to putatively identify the bacteria in each classification unit and the corresponding OTU.

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Supplementary Table 1. Comparisons of background information between participants with mild cognitive impairment (MCI) and those with normal cognition (NC)

	MCI (n = 55)	NC (n = 19)	p
Demographics			
Age, y	77, 70−81	69, 59−75	0.002
Sex, female, *n* (%)	26 (47.3)	9 (47.4)	1.000
Education, y	12, 9−12	12, 12−14	0.068
Body mass index, kg/m^2	22.6, 21.2−24.2	22.3, 20.7−23.9	0.524
Risk factors			
Hypertension, *n* (%)	40 (72.7)	7 (36.8)	0.011
Diabetes mellitus, *n* (%)	6 (10.9)	3 (15.8)	0.686
Dyslipidemia, *n* (%)	24 (43.6)	7 (36.8)	0.788
CKD, *n* (%)	16 (29.1)	5 (26.3)	1.000
Smoking habits, *n* (%)	22 (40.0)	5 (26.3)	0.408
Alcohol consumption, *n* (%)	23 (41.8)	12 (63.2)	0.120
APOE ε4 carrier, *n* (%)	13 (23.6)	3 (15.8)	0.747
Comprehensive geriatric assessment			
Barthel Index	100, 100−100	100, 100−100	0.261
IADL impairment, *n* (%)	23 (41.8)	2 (10.5)	0.013
DBDS	9, 4–14	3, 0–10	0.002
GDS	2, 1–4	4, 2–6	0.051
Vitality index	10, 9–10	10, 10–10	0.098
History of fall in a year, *n* (%)	23 (43.4)	6 (31.6)	0.424
Gait speed, m/s	1.14, 0.91−1.31	1.17, 0.89−1.26	0.968
MNA-SF	13, 11−13	12, 11−13	0.585
Cognitive function			
MMSE	26, 23−28	29, 28−30	<0.001
CDR-SB	2, 1−3	0, 0−0.5	<0.001
ADAS-cog	7.8, 5.3–12.1	5.7, 3.5–8.3	0.018
RCPM	28, 24–32	32, 30–34	0.003
FAB	11, 10–13	14, 12–16	<0.001
LM-WMSR I	10, 6–15	19, 8–24	0.007
LM-WMSR II	3, 0–10	12, 4–18	0.003

Brain MRI findings			
Total SVD score ≥ 2, *n* (%)	11 (30.1)	2 (5.3)	0.006
SLI, *n* (%)	4 (7.3)	1 (5.3)	1.000
Severe WMH, *n* (%)	18 (32.7)	1 (5.3)	0.030
CMB, *n* (%)	9 (16.4)	2 (10.5)	0.718
BG-EPVS ≥ 2, *n* (%)	14 (25.5)	0	0.015
CSO-EPVS ≥ 3, *n* (%)	22 (40.0)	2 (10.5)	0.023
VSRAD	0.9, 0.7–1.6	0.7, 0.4–1.0	0.093
Arterial stiffness			
Ankle brachial index	1.10, 1.05−1.14	1.14, 1.07−1.16	0.134
Pulse wave velocity, m/s	19.0, 16.1−23.3	17.0, 13.8−21.0	0.094
Laboratory findings			
NfL, pg/mL	23.8, 18.7−33.3	19.0, 13.8−20.8	<0.001
F/B ratio	1.22 (0.68-2.10)	1.65 (1.11-3.19)	0.048
Enterotype I, *n* (%)	31 (56.4)	5 (26.3)	0.033

Data are presented as medians, interquartile ranges or number of patients (%).

The Wilcoxon rank-sum test and χ^2 test were used.

ADAS-cog, Alzheimer's Disease Assessment Scale-Cognitive Subscale; *APOE*, apolipoprotein E; BG-EPVS, enlarged perivascular spaces in the basal ganglia; CDR-SB, Clinical Dementia Rating-Sum of Boxes; CKD, chronic kidney disease; CMB, cerebral microbleed; CSO-EPVS, enlarged perivascular spaces in the centrum semiovale; DBDS, Dementia Behavior Disturbance Scale; FAB, Frontal Assessment Battery; F/B ratio, firmicutes/bacteroidetes ratio; GDS, Geriatric Depression Scale; IADL, instrumental activities of daily living; LM-WMSR, Logical Memory subtests I and II of the Wechsler Memory Scale-Revised; MMSE, Mini-Mental State Examination; MNA-SF, Mini-Nutritional Assessment-Short Form; MRI, magnetic resonance imaging; NfL, neurofilament light chain; RCPM, Raven's Coloured Progressive Matrices; SLI, silent lacunar infarct; SVD, small vessel disease; VSRAD, voxel-based specific regional analysis system for Alzheimer's disease; WMH, white matter hyperintensity.

Supplementary Table 2. Comparisons of background information between participants with severe enlarged perivascular spaces in the basal ganglia (BG-EPVS) and those without

	BG-EPVS ≥ 2 (*n* = 14)	BG-EPVS < 2 (*n* = 60)	*p*
Demographics			
Education, y	9, 9−12	12, 11−14	0.002
Body mass index, kg/m^2	22.4, 20.8−24.2	22.6, 21.1−24.1	0.852
Systolic BP, mmHg	151, 127−165	143, 124−166	0.820
Diastolic BP, mmHg	85, 71−98	82, 71−89	0.605
Risk factors			
Dyslipidemia, *n* (%)	5 (35.7)	26 (43.3)	0.766
Smoking habits, *n* (%)	2 (14.3)	25 (41.7)	0.069
Alcohol consumption, *n* (%)	5 (35.7)	30 (50.0)	0.386
APOE ε4 carrier, *n* (%)	3 (21.4)	13 (21.7)	1.000
Comprehensive geriatric assessment			
Barthel Index	100, 100−100	100, 100−100	0.697
IADL impairment, *n* (%)	7 (50.0)	18 (30.0)	0.211
GDS	3, 0−5	3, 1−5	0.503
Vitality index	9, 9−10	10, 9−10	0.072
History of fall in a year, *n* (%)	5 (38.5)	24 (40.7)	1.000
MNA-SF	13, 10−13	13, 11−13	0.777
Cognitive function			
ADAS-cog	9.4, 4.7−12.7	7.0, 4.9−10.6	0.396
LM-WMSR I	9, 5−11	13, 7−21	0.072
LM-WMSR II	3, 1−7	7, 1−13	0.123

Data are presented as medians, interquartile ranges, or number of patients (%).

The Wilcoxon rank-sum test and χ^2 test were used.

Note that participants with severe BG-EPVS were defined as presenting with enlarged perivascular spaces in the basal ganglia (scores ≥ 2 on the basis of an MRI scan at the level of the basal ganglia).

ADAS-cog, Alzheimer's Disease Assessment Scale-Cognitive Subscale; *APOE*, apolipoprotein E; BG-EPVS, enlarged perivascular spaces in the basal ganglia; BP, blood pressure; CDR-SB, Clinical Dementia Rating-Sum of Boxes; CKD, chronic kidney disease; CMB, cerebral microbleed; CSO-EPVS, enlarged perivascular spaces in the centrum semiovale; DBDS,

Dementia Behavior Disturbance Scale; FAB, Frontal Assessment Battery; F/B ratio, firmicutes/bacteroidetes ratio; GDS, Geriatric Depression Scale; IADL, instrumental activities of daily living; LM-WMSR, Logical Memory subtests I and II of the Wechsler Memory Scale-Revised; MMSE, Mini-Mental State Examination; MNA-SF, Mini-Nutritional Assessment-Short Form; MRI, magnetic resonance imaging; NfL, neurofilament light chain; RCPM, Raven's Coloured Progressive Matrices; SLI, silent lacunar infarct; SVD, small vessel disease; VSRAD, voxel-based specific regional analysis system for Alzheimer's disease; WMH, white matter hyperintensity.

Supplementary Table 3. Comparisons of background information between participants with severe enlarged perivascular spaces in the centrum semiovale (CSO-EPVS) and those without

	CSO-EPVS ≥ 3 (n = 24)	CSO-EPVS < 3 (n = 50)	*p*
Demographics			
Age, y	78, 73−82	74, 67−79	0.006
Sex, female, *n* (%)	11 (45.8)	24 (48.0)	1.000
Education, y	12, 9−14	12, 9−13	0.745
Body mass index, kg/m^2	22.1, 20.9−23.4	23.0, 21.2−24.2	0.353
Risk factors			
Hypertension, *n* (%)	15 (62.5)	32 (64.0)	1.000
Diabetes mellitus, *n* (%)	3 (12.5)	6 (12.0)	1.000
Dyslipidemia, *n* (%)	8 (33.3)	23 (46.0)	0.327
CKD, *n* (%)	8 (33.3)	13 (26.0)	0.586
Smoking habits, *n* (%)	5 (20.8)	22 (44.0)	0.072
Alcohol consumption, *n* (%)	11 (45.8)	24 (48.0)	1.000
APOE ε4 carrier, *n* (%)	5 (20.8)	11 (22.0)	1.000
Comprehensive geriatric assessment			
Barthel Index	100, 100−100	100, 100−100	0.919
IADL impairment, *n* (%)	12 (50.0)	13 (26.0)	0.065
DBDS	12, 5–18	6, 2–12	0.007
GDS	3, 1–5	3, 2–6	0.459
Vitality index	10, 9–10	10, 9–10	0.632
History of fall in a year, *n* (%)	8 (33.3)	21 (43.8)	0.453
Gait speed, m/s	1.12, 0.70−1.27	1.16, 0.94−1.31	0.366
MNA-SF	13, 12−13	13, 11−13	0.850
Cognitive function			
MMSE	26, 24−29	28, 23−29	0.406
CDR-SB	1.3, 0.5−2.5	1.0, 0.4−2.6	0.211
ADAS-cog	8.0, 4.3–12.7	7.3, 5.2–10.9	0.831
RCPM	26, 22–32	30, 27–34	0.016
FAB	11, 10–13	13, 10–14	0.083
LM-WMSR I	9, 5–15	13, 7–21	0.088
LM-WMSR II	3, 0–9	7, 2–13	0.129

Brain MRI findings			
Total SVD score ≥ 2, *n* (%)	5 (20.8)	8 (16.0)	0.746
SLI, *n* (%)	2 (8.3)	3 (6.0)	0.657
Severe WMH, *n* (%)	8 (33.3)	11 (22.0)	0.395
CMB, *n* (%)	4 (16.7)	7 (14.0)	0.740
BG-EPVS ≥ 2, *n* (%)	9 (37.5)	5 (10.0)	0.009
VSRAD	0.99, 0.70–1.63	0.74, 0.54–1.46	0.233
Arterial stiffness			
Ankle brachial index	1.07, 1.04−1.17	1.11, 1.07−1.15	0.495
Pulse wave velocity, m/s	20.4, 16.0−23.6	17.3, 15.5−21.1	0.125
Laboratory findings			
NfL, pg/mL	24.9, 21.1−36.4	20.6, 15.2−25.3	0.009
F/B ratio	1.26 (0.72-2.65)	1.30 (0.82-2.06)	0.926
Enterotype I, *n* (%)	12 (50.0)	24 (48.0)	1.000

Data are presented as medians, interquartile ranges or number of patients (%).

The Wilcoxon rank-sum test and χ^2 test were used.

Note that participants with severe CSO-EPVS were defined as presenting with enlarged perivascular spaces in the centrum semiovale (scores ≥3 on the basis of an MRI scan at the level of the centrum semiovale).

ADAS-cog, Alzheimer's Disease Assessment Scale-Cognitive Subscale; APOE, apolipoprotein E; BG-EPVS, enlarged perivascular spaces in the basal ganglia;

CDR-SB, Clinical Dementia Rating-Sum of Boxes; CKD, chronic kidney disease; CMB, cerebral microbleed; CSO-EPVS, enlarged perivascular spaces in the centrum semiovale; DBDS, Dementia Behavior Disturbance Scale; FAB, Frontal Assessment Battery; F/B ratio, firmicutes/bacteroidetes ratio; GDS, Geriatric Depression Scale; IADL, instrumental activities of daily living; LM-WMSR, Logical Memory subtests I and II of the Wechsler Memory Scale-Revised; MMSE, Mini-Mental State Examination; MNA-SF, Mini-Nutritional Assessment-Short Form; MRI, magnetic resonance imaging; NfL, neurofilament light chain; RCPM, Raven's Coloured Progressive Matrices; SLI, silent lacunar infarct; SVD, small vessel disease; VSRAD, voxel-based specific regional analysis system for Alzheimer's disease; WMH, white matter hyperintensity.

Supplementary Table 4. Univariable and multivariable ordinal logistic regression analyses for severity of enlarged perivascular spaces in the centrum semiovale (CSO-EPVS)

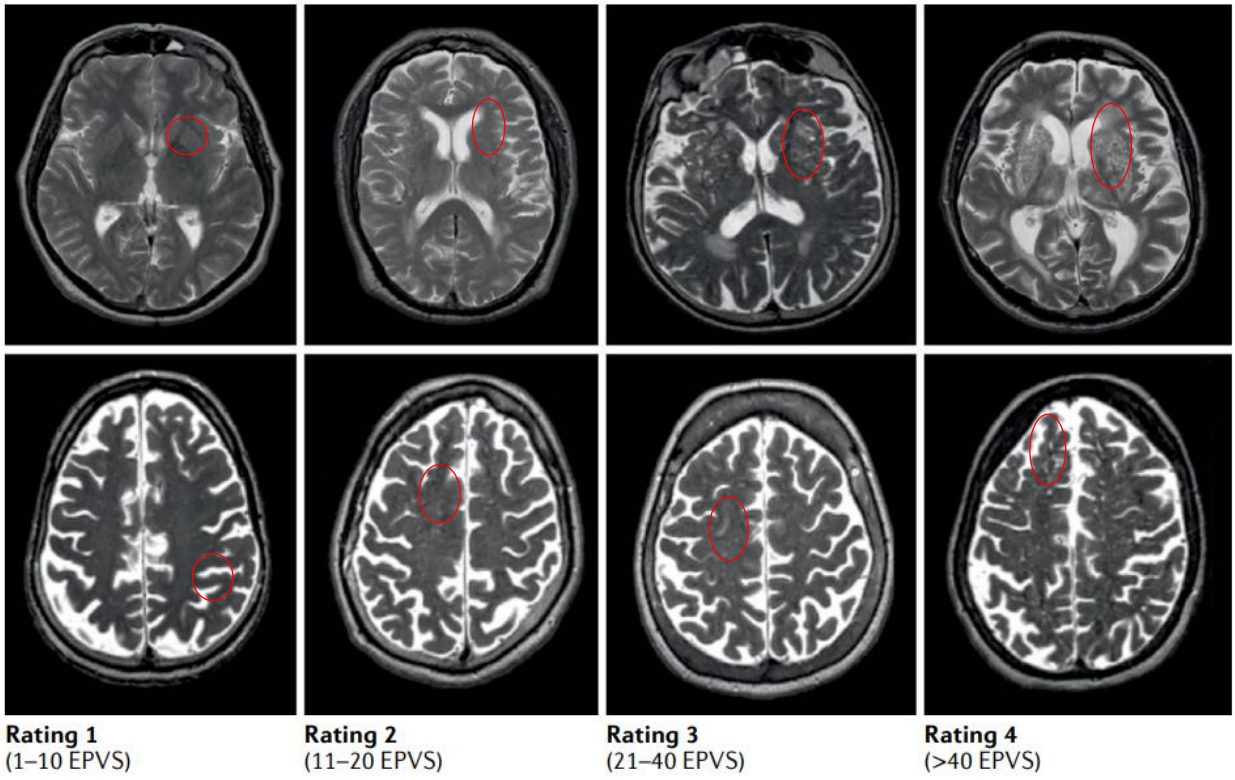
	Univariable	Multivariable
PWV, by 1 m/s	1.09 (0.99−1.21)	1.07 (0.95−1.20)
Age, years	1.08 (1.03−1.14) **	1.10 (1.03−1.16) **
Female sex	0.75 (0.31−1.80)	0.77 (0.31−1.93)
Hypertension	0.94 (0.38−2.33)	0.39 (0.14−1.11)
Diabetes mellitus	0.83 (0.22−3.20)	0.68 (0.17−2.71)
Enterotype I	0.96 (0.40−2.29)	0.70 (0.27−1.80)

Data was presented as odds ratio (95% confidential interval).

* $p < 0.05$, ** $p < 0.01$

Adjusted for brachial-ankle PWV, age, sex, hypertension, diabetes mellitus, and the gut microbiota (enterotype I).

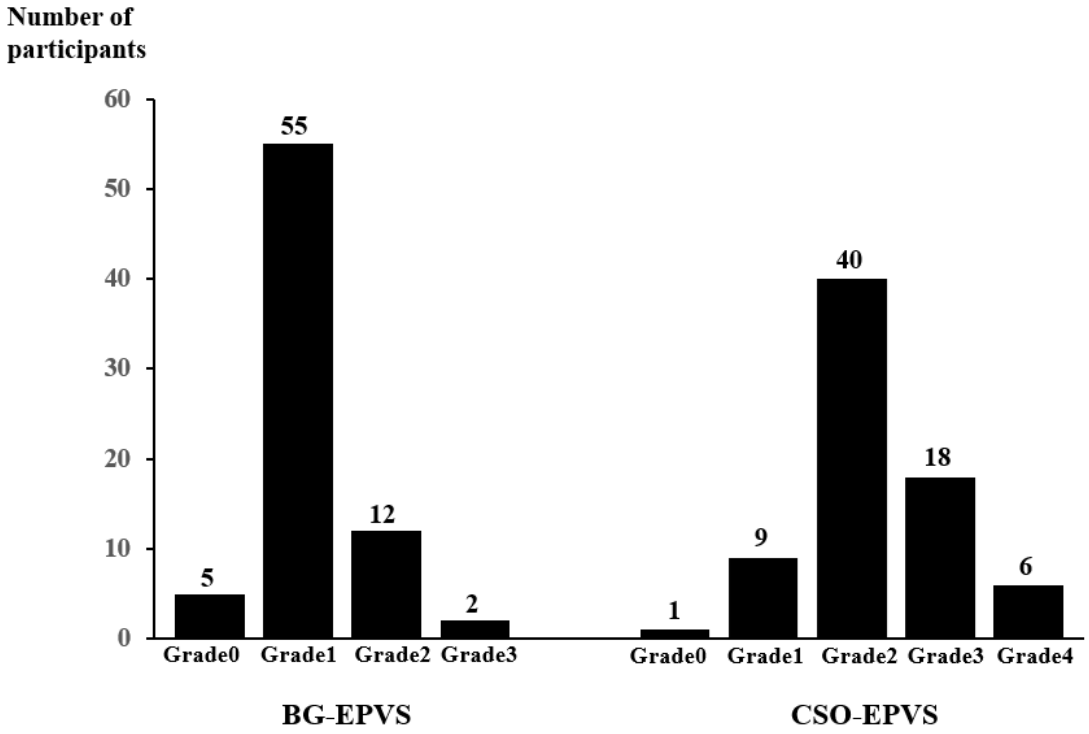
CI, confidence interval; CSO-EPVS, enlarged perivascular spaces in the centrum semiovale; OR, odds ratio; PWV, pulse wave velocity.



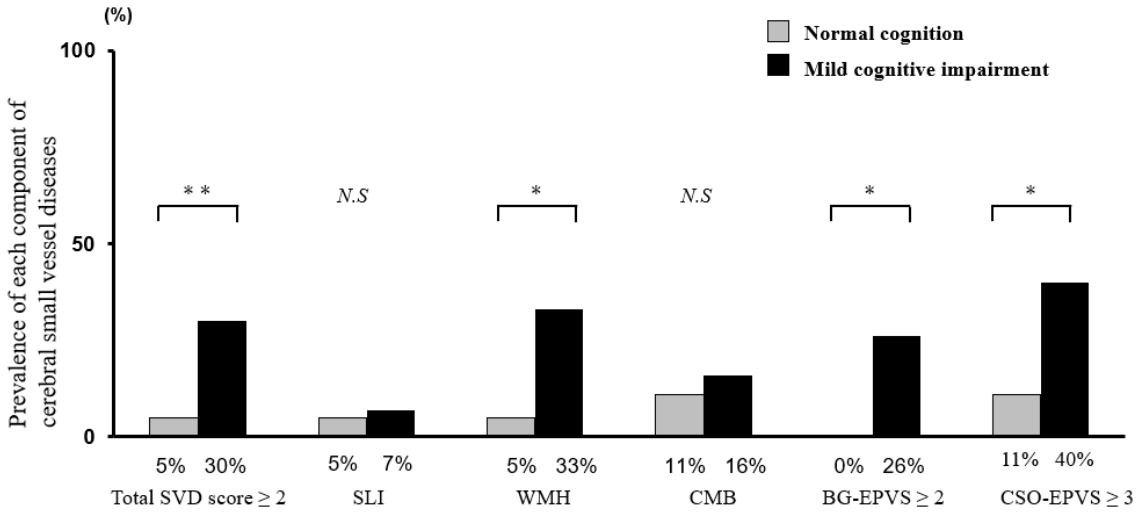
Supplementary Figure 1. Perivascular spaces visualized with MRI in humans.[31]

T2-weighted MRI scans that illustrate different extents of perivascular space visibility in the basal ganglia (top row) and the centrum semiovale (bottom row). Visual rating (1–4) scores for enlarged perivascular spaces (EPVS) are indicated at the bottom.

The red oval region shows EPVS. Adapted from "Perivascular spaces in the brain: anatomy, physiology and pathology", by Wardlaw JM, 2020, Nat Rev Neurol 16, p. 137-153. Adapted with permission.



Supplementary Figure 2. The number of participants per BG-EPVS or CSO-EPVS grades (0-4) in the present study.



Supplementary Figure 3. Prevalence of each component of cerebral small vessel disease (SVD) stratified by cognitive function

Here, the prevalence rates of each component of cerebral small vessel disease (SVD) are plotted relative to cognitive function. The following components of cerebral SVD are shown: total SVD score ≥ 2, silent lacunar infarct (SLI), white matter hyperintensity (WMH), cerebral microbleeds (CMB), and enlarged perivascular spaces in the basal ganglia (BG-EPVS scores ≥ 2) and in the centrum semiovale (CSO-EPVS scores ≥ 3). Cognitive function stratified into normal cognition and mild cognitive impairment. Analyses were performed using the χ^2 test. * $p < 0.05$, ** $p < 0.01$, N.S; not significant.