SUPPLEMENTAL MATERIAL

Arterial Elasticity, Endothelial Function and Intracranial Vascular Health: A Multimodal MRI Study

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Supplementary Methods

1. Eligibility criteria of the BRAVE-1 study

- Inclusion criteria:
  1) Males aged over 55 years;
  2) A clinical diagnosis of primary hypertension which was made at least 2 months before enrollment;
  3) Education level above primary school.
- Exclusion criteria:
  1) History of neurologic diseases (including but not limited to stroke, Alzheimer's disease or Parkinson's disease) or brain trauma;
  2) History of cardiovascular diseases, including coronary artery disease, valvular heart disease, cardiomyopathy, atrial fibrillation, atrioventricular block (II or III degree) or peripheral artery disease;
  3) Psychological disorders, including but not limited to depression and schizophrenia;
  4) Unable to read or write due to visual impairment or any other cause;
  5) Contraindications of MRI;
  6) Receiving hemodialysis or peritoneal dialysis due to end-stage kidney disease or history of renal transplant;
  7) Life expectancy <1 year due to malignancy or other conditions;
  8) Any other conditions that preclude the subject from taking the tests required by the study or considered as inappropriate for participation by research staffs.

2. MRI Review Process

- **Intracranial atherosclerosis**: 3D T1-weighted FSE vessel wall images were used to identify intracranial atherosclerotic plaque. The images were checked for image quality (IQ) first and only those with sufficient IQ were reviewed for further analysis. Images were considered to have sufficient quality if lumen and outer wall boundaries are both visible in the cerebral arteries included in the review. Images with low SNR and/or severe artifacts that obscure vessel wall boundaries in these cerebral artery segments were excluded. An image reader, who was certified by our internal vessel wall image review training program, reviewed all the images using RadiAnt Dicom Viewer (Medixant Inc., Poland) without knowledge of the clinical information. A plaque was defined as visible focal wall thickening regardless of luminal stenosis on angiography. The major segments of the following arteries were assessed: intracranial internal carotid artery, middle cerebral artery (M1 segments), anterior cerebral artery (A1 segments), posterior cerebral artery (P1 segments) and basilar artery. Multi-Planar Reconstruction was used to reconstruct images both parallel and perpendicular to the longitudinal axis when necessary. The TOF images were then used to determine if luminal stenosis was present for each plaque. Stenosis was defined as luminal narrowing of at least 30% as measured and compared to the proximal non-stenotic slice. The results were peer-reviewed by another image reader with over ten years' experience in vessel wall image review.
- **Vascular rarefaction**: The TOF angiography images were analyzed using the iCafe (intraCranial Artery Feature Extraction) tool. The iCafe is a recently custom-developed technique for quantitative analysis of intracranial arterial morphological features (e.g. number of branches, vascular tortuosity). In the current study, we used the "artery branch number", which is considered as reflection of vascular rarefaction, as the primary outcome since we have demonstrated that it is a prominent feature that

changes in the process of aging. A trained reader used the software to process the images. Briefly, MRA images were resampled using bi-cubic interpolation for isotropic resolution in 3D space. Artery regions were then traced using an open-curve active contour model and the reader manually corrected the traces when needed. The arteries were then labelled with anatomical names (e.g. right internal carotid artery, left M1 segment of middle cerebral artery and etc.). The final results were peer-reviewed by the software developer. An artery branch was defined as a trace which start from a bifurcation and end in another bifurcation or termination in a vascular group. The branch number was calculated as the total number of artery branches excluding the major large segments (e.g. internal carotid arteries, vertebral arteries, basilar artery, M1, P1 and A1). Artery length is the end-to-end distance of the centerline of an artery. Total distal vessel length was calculated as the sum of all the artery length excluding the major large segments.

- **Brain perfusion**: Cerebral blood flow (CBF) map was calculated from 3D pcASL images, which include the perfusion-weighted images and proton density images, by using the recommended hemodynamic model. Co-registration was performed between the ASL images and the MP-RAGE anatomical images using Statistical Parametric Mapping (SPM) toolbox. For each subject, a mask of whole brain grey matter was generated by performing segmentation of the brain on the MP-RAGE images, and then a mean grey matter CBF value was calculated from those voxels within the mask. White matter CBF was not assessed since it is low and may not be accurately estimated using ASL.
- **White matter hyperintensity**: The FLAIR and 3D T1-weighted FSE images were used to quantify intracranial WMH volume. The processing steps followed similar methods from Roura et al. using their publicly available MATLAB Multiple Sclerosis Lesion Segmentation (SLS) toolbox, an extension for the Statistical Parametric Mapping (SPM) software toolbox. FLAIR and 3D T1-weighted FSE images were first denoised using the anisotropic diffusion filter provided by the Insight Toolkit (ITK)

software library and corrected for intensity inhomogeneities using the non-parametric, non-uniform intensity (N3) normalization method[6]. The FLAIR images were then co-registered to the 3D T1-weighted FSE images where the Montreal Neurological Institute (MNI) space, provided by SPM, was used as the target space. Once co-registered, SPM was used to mask and generate tissue probability maps of the 3D T1-weighted FSE image then used the brain mask to skull-strip the FLAIR image. The 3D T1-weighted FSE grey matter (GM) tissue probability map was then used to compute the intensity distribution of the GM in the FLAIR image to detect hyperintense outliers for segmentation. The segmentation results were reviewed to manually correct errors and exclude lesions detected in the cerebellum and brainstem, then quantified volume using fslstats from the FMRIB Software Library (FSL). Similarly, Intracranial volume was calculated by summing the tissue probability maps using fslstats.

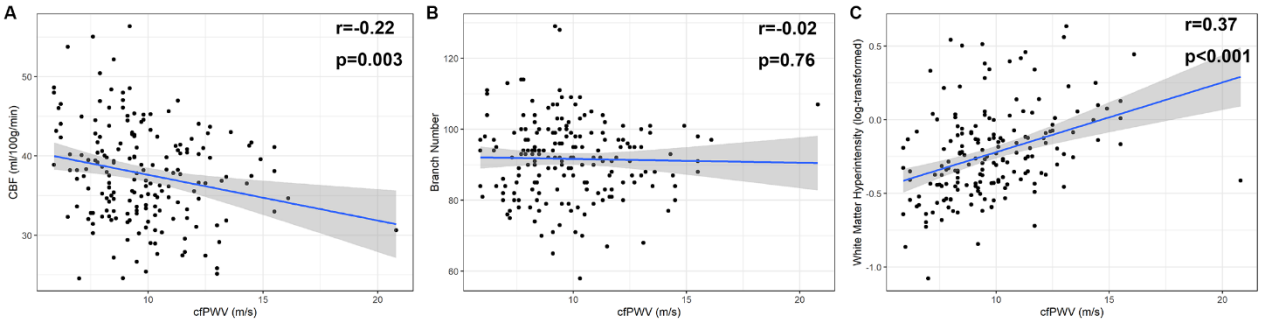
Table S1. Imaging Protocols for the BRAVE-1 Study

	3D T1-FSE	3D TOF	3D ASL	3D MP-RAGE	2D FLAIR
FOV, cm^3	18*18*4.8	22*19.8*9.8	24*24*12	22*22*16.5	24*24*12.8
FOV orientation	Coronal	Axial	Axial	Sagittal	Axial
Resolution*, mm^3	0.625*0.625*0.6	0.86*0.77*1.2	1.875*1.875*4	1.1*1.1*1.1	0.83*1.07*5
TR/TE, ms	900.0/20.0	25.0/3.4	4521.0/9.8	5.6/1.6	9000.0/120.0
Flip Angle	90	20	155	15	90
Number of Signal Averages	1.0	1.0	3	1.5	1
Acceleration Factor	1.0	2.0	1.0	1.0	1
Scan duration	9:38	6:13	4:22	4:09	3:37

* Acquired resolutions.

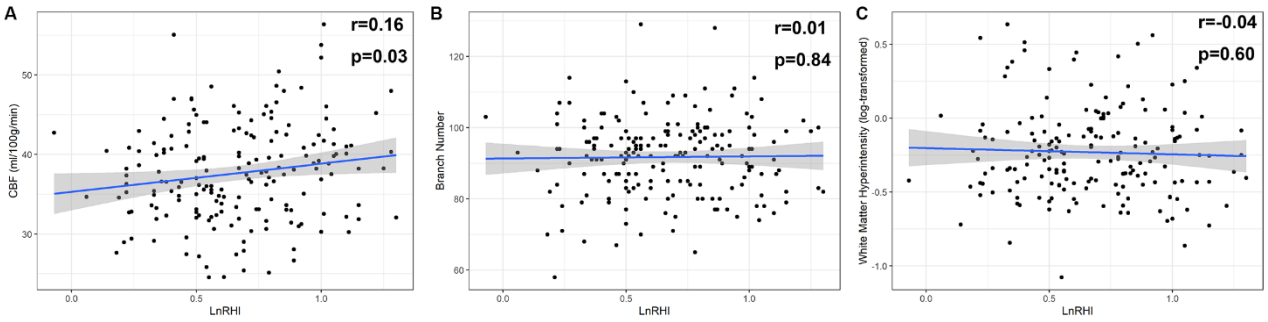
Supplementary Results

Figure S1. Scatterplots for correlations of cfPWV to CBF, Arterial Branch Number and WMH



A: Correlation of cfPWV to CBF; B: Correlation of cfPWV to branch number (vascular rarefaction analyzed from iCafe); C: Correlation of cfPWV to WMH (WMH volume is expressed as (percentage of intracranial volume) × 100, and is log-transformed). Abbreviations: CBF, cerebral blood flow; cfPWV, carotid-femoral pulse wave velocity; WMH, white matter hyperintensity.

Figure S2. Scatterplots for correlations of LnRHI to CBF, Arterial Branch Number and WMH



A: Correlation of LnRHI to CBF; B: Correlation of LnRHI to branch number (vascular rarefaction analyzed from iCafe); C: Correlation of LnRHI to WMH (WMH volume is expressed as (percentage of intracranial volume) × 100, and is log-transformed). Abbreviations: CBF, cerebral blood flow; LnRHI, natural log transformed reactive hyperemia index; WMH, white matter hyperintensity.