

Supplementary Methods

DNA Methylation and Protein Markers of Chronic Inflammation and their Associations with Brain and Cognitive Ageing

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Supplementary Methods

Neuroimaging

LBC1936 Image Acquisition and MRI parameters

Participants underwent whole brain structural and high angular resolution 2mm isotropic voxel diffusion tensor MRI (DT-MRI) using a 1.5T GE Sigma Horizon HDxt clinical scanner (General Electric, Milwaukee, WI, USA) operating in research mode using a shelf-shielding gradient set with maximum gradient of 33 mT/m, and an 8 channel phased-array head coil. Mean age at scanning was 72.7 (SD 0.7) years. The DT-MRI protocol comprised single-shot spin-echo echo-planar (EP) diffusion weighted volumes ($b = 1000 \text{ s mm}^{-2}$) acquired in 64 noncollinear directions, alongside 7 T2-weighted EP volumes ($b = 0 \text{ s mm}^{-2}$). This protocol was run with 72 contiguous axial slices with a field of view of $256 \times 256 \text{ mm}$, an acquisition matrix of 128×128 and 2mm isotropic voxels. Repetition and echo times were 16.5 s and 95.5 ms, respectively. The full imaging protocol for the LBC1936 has been published previously¹.

Freesurfer Quality Control

For cortical volume and cortical thickness measurements, participants with a T1-weighted scan and complete inflammation data were selected (n=666). Acquired volumes were then processed in FreeSurfer v5.1^{2,3}. This involved segmentation of each volume, identifying brain tissue types, followed by parcellation of cortical grey matter into 34 regions per hemisphere, according to the Desikan- Killiany atlas⁴. Quality control involved visually assessing each image output for segmentation and parcellation errors, which were then corrected manually; segmentations with errors that could not be corrected were excluded (n = 37), bringing the total eligible to 629 participants. Participants were then excluded if they had self-reported history of neurodegeneration or signs of cognitive impairment (n=69); additionally, participants with high inflammation levels indicative of infection or illness were excluded (n=32). After exclusions, a total of 521 participants had complete inflammation, cognitive neuroimaging and relevant health data; see flow diagram of attrition, figure 2.

Structural MRI volumetric analysis

Intracranial volume (ICV) was semi-automatically extracted on T2*W images using the Object Extraction Tool in Analyze 9.0 (Mayo Clinic, Analyze 9.0. AnalyzeDirect, Inc. Mayo Clinic) followed by manual editing to remove erroneous structures as described in (Valdés Hernández et al, 2012). In order to estimate total brain tissue volume, cerebrospinal fluid (CSF), venous sinuses and meninges were subtracted from ICV. The CSF and these non-brain tissue structures were extracted

using the combination of T2*W and FLAIR sequences and the MCMxxxVI method as described in⁵. Quantitative estimates of WMH were estimated using the same MCMxxxVI method⁵ fusing T2*W and FLAIR images. False positives and old infarcts were visually identified and removed manually. Total brain (TB), grey matter (GM), normal-appearing white matter (NAWM) volumes were also segmented using this semi-automated multi-spectral technique⁵. WMH was log-transformed, and to control for overall size of the head, we present all MRI measures (TB, GM, NAWM, WMH) as a percentage of ICV in subsequent analyses.

White matter tract analysis

White matter connectivity data – measures of fractional anisotropy (FA) and mean diffusivity (MD) – were estimated using the BEDPOSTX/ProbTrackX algorithm in FSL (<https://fsl.fmrib.ox.ac.uk>) and 12 major tracts of interest were segmented using TractoR (<https://www.tractor-mri.org.uk>): the genu and splenium of the corpus callosum; arcuate fasciculi; anterior thalamic radiation; rostral cingulum; uncinate fasciculi; inferior longitudinal fasciculi. Tract-average white matter FA and MD were derived as the average of all voxels contained within the resultant tract maps, as described previously⁶. A general factor of FA (g_{FA}) and MD (g_{MD}) was derived for each participant from the first unrotated principal component of a principal components analysis (PCA) on twelve of the white matter tracts FA and MD values; these general factors reflect common microstructural properties across main white matter pathways and capture the common variance in white matter integrity⁷. Participants with up to 2 missing values from specific tracts had data replaced with the mean value for that tract. Details of individual test loadings are provided in the supplementary document (eTable 14).

Cognitive variables

All participants in the LBC1936 underwent a detailed battery of standardised cognitive tests; from these, participant scores for three distinct cognitive domains (visuospatial ability, processing speed and verbal memory) were created, based upon well-fitting, hierarchical structural equation models tested in our previously published work⁸⁻¹⁰.

Visuospatial ability was assessed by performance on Matrix Reasoning and Block Design from the Wechsler Adult Intelligence Scale III^{UK} (WAIS III^{UK})¹¹ and the sum of Spatial Span Forward and Backward from the Wechsler Memory Scale III^{UK} (WMS III^{UK})¹². Processing Speed was measured with Symbol Search and Digit Symbol Substitution from the WAIS III^{UK}, Visual Inspection Time¹³ and Four-Choice Reaction Time¹⁴. Verbal Memory ability was measured using Logical Memory (sum of immediate and delayed) and Verbal Paired Associates (sum of immediate and delayed) from the WMS III^{UK}, and Digit Span Backwards from the WAIS III^{UK}.

We also derived a single general fluid-type cognitive ability score (g_f) for each participant from the first un-rotated principal component of a principal components analysis on six of the Wechsler Adult Intelligence Scale-III tests – an approach that has been used both in this cohort¹⁵ and in others¹⁶. This fluid-type cognitive ability measure (g_f) represents the capacity to perform basic information processing and extemporary thinking tasks, rather than those involving acquired knowledge or experience. Scores on different tests of fluid-type cognitive ability are typically highly correlated¹⁷, indicative of a latent general cognitive ability factor, and g_f has shown to be particularly sensitive to the effects of ageing⁸. Relevant cognitive tests and individual weightings of g_f and the latent variables of processing speed, visuospatial ability and verbal memory can be found in supplementary document (eTable 3)

Lifestyle variables

All subjects were interviewed and tested individually by a trained psychologist and a research nurse during a visit to the Wellcome Trust Clinical Research Facility (<http://www.wtcrf.ed.ac.uk>), Western General Hospital, Edinburgh. Trained research nurses measured height and weight as part of a physical examination using a standardized protocol. Height (in centimeters) was measured with a SECA stadiometer on individuals not wearing shoes. The research nurses measured weight (in kilograms) for individuals without outer clothing or shoes using electronic SECA scales with a digital readout. BMI was calculated as weight (in kilograms) divided by height squared (in square meters). The clinical interview then assessed the participant's self-reported health and lifestyle: cardiovascular disease history (CVD); hypertension; diabetes; smoking status (coded as current smoker [1] versus ex/non-smoker [0]) and current alcohol use (alcohol units per week).

eTable 1. Participant characteristics

Class	Variable	Units	mean	SD
Demographics	sex	M: F	358 : 322	-
	Age	years	72.494	0.716
Inflammation	DNAm CRP	Std units	-0.018	0.001
	serum CRP (high sensitivity)	mg/L	2.016	1.92
Neuroimaging	TB	cm ³	992.0	89.8
	GM	cm ³	473.5	45.1
	NAWM	cm ³	477.0	50.67
	WMH	cm ³	8.800	1.31
	Cortical thickness	mm	3.130	0.15
	gFA	Std units	0.040	0.97
	gMD	Std units	-0.023	0.97
	Genu of corpus callosum	Std units	0.376	0.046
	Splenium of corpus callosum	Std units	0.511	0.066
	Left Arcuate Fasciculus	Std units	0.436	0.039
Fractional anisotropy	Right Arcuate Fasciculus	Std units	0.415	0.039
	Left Anterior Thalamic Radiation	Std units	0.324	0.032
	Right Anterior Thalamic Radiation	Std units	0.335	0.035
	Left Rostral Cingulum	Std units	0.437	0.058
	Right Rostral Cingulum	Std units	0.410	0.047
	Left Uncinate fasciculus	Std units	0.325	0.034
	Right Uncinate fasciculus	Std units	0.320	0.031
	Left Inf. Longitudinal Fasciculus	Std units	0.386	0.051
	Right Inf. Longitudinal Fasciculus	Std units	0.377	0.048
	Genu of corpus callosum	Std units	798.7	78.3
Mean diffusivity	Splenium of corpus callosum	Std units	809.0	123.6
	Left Arcuate Fasciculus	Std units	658.5	50.4
	Right Arcuate Fasciculus	Std units	644.8	50.6
	Left Anterior Thalamic Radiation	Std units	756.5	63.9
	Right Anterior Thalamic Radiation	Std units	739.1	76.2
	Left Rostral Cingulum	Std units	636.1	45.3
	Right Rostral Cingulum	Std units	622.4	44.1
	Left Uncinate fasciculus	Std units	764.6	57.7
	Right Uncinate fasciculus	Std units	760.6	49.0
	Left Inf. Longitudinal Fasciculus	Std units	772.7	105.4
Cognitive	Right Inf. Longitudinal Fasciculus	Std units	757.9	97.1
	gf	Std units	0.052	0.975
	processing speed	Std units	0.058	0.947
	visuospatial ability	Std units	0.048	0.997
Lifestyle	verbal memory	Std units	0.04	0.972
	hypertension	% Yes	53%	-
	CVD	% Yes	29%	-
	diabetes	% Yes	10%	-
	alcohol units per week	Std units	14.2	14.9
	smokers	% Yes	8%	-
	ex smokers	% Yes	44%	-
	BMI	Kg/m ²	27.8	4.25
	anti-inflammatory medication	% Yes	7.2%	

TB: total brain, GM: grey matter, NAWM: normal-appearing white matter, WMH: white matter hyperintensity, gf, general cognitive ability; gFA: general fractional anisotropy, gMD: general mean diffusivity

eTable 2. CpG sites and relative weights (from Lighthart et al. 2016) used to generate DNA CRP score

<i>CpG</i>	<i>Gene</i>	Beta (discovery sample)
cg06126421	<i>TUBB</i>	-0.0052
cg06690548	<i>SLC7A11</i>	-0.0048
cg10636246	<i>AIM 2 & IFI16</i>	-0.0069
cg18181703	<i>SOCS3</i>	-0.0053
cg19821297	<i>DNASE2</i>	-0.0051
cg25325512	<i>FGD2</i>	-0.0051
cg27023587	<i>HEATR6</i>	-0.005

eTable 3: Test loadings for general factors of cognitive ability measures

<i>Cognitive test</i>	<i>gf*</i>	processing speed	visuospatial ability	verbal memory
digit-span backwards	0.634	-	-	0.608
symbol search	0.746	0.816	-	-
digit-symbol coding	0.739	0.834	-	-
matrix reasoning	0.691	-	0.796	-
letter-number sequencing	0.708	-	-	-
block design	0.71	-	0.839	-
Inspection Time	-	0.628	-	-
Four choice reaction	-	-0.773	-	-
Verbal Paired Associates	-	-	-	0.809
Logical Memory	-	-	-	0.827
Spatial Span	-	-	0.739	-
Proportion of variance	0.498	0.588	0.628	0.569

*Note** the tests used to generate a general score of cognitive ability were used to facilitate comparison with previous work by Stevenson et al. (2019)

eTable 4. Sensitivity analysis: ancillary effect models; interaction effects between age*inflammation, sex*inflammation and anti-inflammatory drug status*inflammation, for DNAm CRP brain-health associations

Brain health phenotype	DNAm CRP*sex			DNAm CRP*age			DNAm CRP*anti-inflammatory drug use		
	β	SE	pFDR	β	SE	pFDR	β	SE	pFDR
TB	0.073	0.082	0.684	-0.070	0.042	0.276	-0.051	0.119	0.834
GM	0.003	0.086	0.972	-0.038	0.044	0.688	-0.013	0.126	0.972
NAWM	0.080	0.086	0.675	-0.013	0.044	0.932	0.127	0.124	0.652
WMH	0.045	0.087	0.828	-0.049	0.045	0.631	-0.138	0.128	0.631
gFA	0.023	0.089	0.834	0.032	0.044	0.774	0.255	0.133	0.168
gMD	-0.049	0.089	0.828	-0.011	0.044	0.932	-0.122	0.132	0.675
g	0.176	0.074	0.060	0.001	0.037	0.972	-0.018	0.118	0.972
visuospatial_ability	0.119	0.074	0.286	-0.009	0.037	0.932	-0.004	0.118	0.972
processing_speed	0.033	0.077	0.834	0.023	0.038	0.828	-0.053	0.115	0.834
verbal_memory	0.047	0.076	0.828	-0.020	0.038	0.828	-0.152	0.119	0.501

eTable 5. Sensitivity analysis: health and lifestyle factors added as covariates into inflammation-brain health regressions.

Brain health metric	(H0)		(H1)		(H2)		(H3)		(H4)		(H5)		(H6)		(H7)	
	Model 1		Fully-adjusted model		hypertension		CVD history		diabetes		BMI		alcohol		smoking	
	β	p(FDR)	β	p(FDR)	β	p(FDR)	β	p(FDR)	β	p(FDR)	β	p(FDR)	β	p(FDR)	β	p(FDR)
TB	-0.197	8.42E-06	-0.137	0.002	-0.196	1.19E-04	-0.194	1.19E-04	-0.191	1.19E-04	-0.183	2.18E-04	-0.197	1.19E-04	-0.167	7.27E-04
GM	-0.200	1.66E-05	-0.153	0.001	-0.192	1.92E-04	-0.199	1.19E-04	-0.194	1.91E-04	-0.182	4.16E-04	-0.200	1.19E-04	-0.179	5.80E-04
NAWM	-0.150	5.47E-04	-0.089	0.052	-0.147	0.004	-0.147	0.003	-0.145	0.004	-0.138	0.007	-0.149	0.003	-0.115	0.027
WMH	0.108	0.017	0.071	0.118	0.096	0.052	0.109	0.030	0.106	0.036	0.097	0.055	0.109	0.030	0.089	0.079
gFA	-0.162	6.97E-04	-0.108	0.022	-0.151	0.003	-0.155	0.003	-0.164	1.98E-03	-0.133	0.013	-0.162	1.98E-03	-0.146	0.007
gMD	0.124	0.010	0.130	0.007	0.121	0.022	0.123	0.020	0.124	0.020	0.141	0.008	0.124	0.020	0.114	0.031
g	-0.158	6.55E-05	-0.101	0.009	-0.154	4.16E-04	-0.154	4.26E-04	-0.150	5.80E-04	-0.148	7.47E-04	-0.158	3.10E-04	-0.129	0.003
visuospatial ability	-0.097	0.014	-0.051	0.189	-0.093	0.030	-0.090	0.034	-0.089	0.037	-0.101	0.023	-0.097	0.027	-0.066	0.137
processing speed	-0.144	4.64E-04	-0.067	0.091	-0.138	2.00E-03	-0.139	2.00E-03	-0.132	0.003	-0.122	0.007	-0.144	1.38E-03	-0.110	0.017
verbal memory	-0.095	0.017	-0.076	0.061	-0.096	0.030	-0.099	0.027	-0.090	0.041	-0.095	0.034	-0.095	0.030	-0.080	0.072

Note: Regression models with added health covariates entered individually. Standardised betas (p values) reported. bold typeface denotes $p < 0.05$ (FDR corrected).

The following regressions are reported:

Brain health phenotype ~ DNAm CRP + age + sex + BMI + smoking + alcohol + diabetes + hypertension + CVD history

eTable 6: FA white matter tract associations with inflammation measures

White matter tract	Side of hemisphere	Serum CRP (model 1)			Epigenetic CRP (model 2)			Δ association magnitudes	
		β	SE	p	β	SE	p	p	
gFA		-0.055	0.045	0.713	-0.162	0.045	0.001	0.045	
Corpus callosum	Genu	-0.016	0.044	0.999	-0.119	0.045	0.008	0.037	
	Splenium	-0.090	0.044	0.302	-0.078	0.044	0.077	0.817	
Arcuate fasciculus	Left	0.002	0.043	0.999	-0.108	0.044	0.014	0.030	
	Right	-0.053	0.045	0.713	-0.150	0.046	0.001	0.062	
Anterior thalamic radiation	Left	-0.036	0.045	0.830	-0.120	0.044	0.007	0.100	
	Right	-0.039	0.043	0.830	-0.126	0.044	0.004	0.084	
Rostral cingulum	Left	-0.006	0.044	0.999	-0.050	0.044	0.253	0.389	
	Right	-0.124	0.045	0.089	-0.140	0.045	0.002	0.753	
Uncinate fasciculus	Left	0.001	0.044	0.999	-0.106	0.045	0.020	0.040	
	Right	-0.034	0.044	0.830	-0.099	0.044	0.025	0.197	
Inferior longitudinal fasciculus	Left	-3.31×10^{-5}	0.043	0.999	-0.032	0.044	0.466	0.531	
	Right	-0.073	0.044	0.496	-0.084	0.043	0.054	0.825	

Note **Supplementary eTable 6.** Results of linear regression analyses of epigenetic CRP score and serum CRP score with white matter tract microstructural metrics (FA). Furthest right column displays difference between association magnitudes of both models, as assessed by Williams' test.

eTable 7: MD white matter tract associations with inflammation measures

White matter tract	Side of hemisphere	Serum CRP (model 1)			Epigenetic CRP (model 2)			Δ association magnitudes	
		β	SE	p	β	SE	p	p	
gMD		-0.025	0.045	0.758	0.124	0.045	0.010		0.056
Corpus callosum	Genu	-0.034	0.044	0.758	0.060	0.045	0.180		0.067
	Splenium	0.056	0.043	0.730	0.079	0.044	0.074		0.650
Arcuate fasciculus	Left	-0.028	0.043	0.758	0.105	0.044	0.016		0.009
	Right	0.018	0.044	0.758	0.111	0.045	0.015		0.075
Anterior thalamic radiation	Left	-0.029	0.045	0.758	0.083	0.044	0.063		0.029
	Right	-0.022	0.043	0.758	0.016	0.044	0.717		0.452
Rostral cingulum	Left	0.074	0.044	0.610	0.078	0.043	0.073		0.940
	Right	0.074	0.044	0.610	0.093	0.044	0.033		0.717
Uncinate fasciculus	Left	-0.015	0.046	0.758	0.046	0.046	0.318		0.243
	Right	0.037	0.043	0.758	0.139	0.044	0.002		0.045
Inferior longitudinal fasciculus	Left	-0.066	0.043	0.610	0.054	0.044	0.220		0.018
	Right	0.022	0.045	0.758	0.013	0.044	0.763		0.868

Note Supplementary eTable 7. Results of linear regression analyses of epigenetic CRP score and serum CRP score with white matter tract microstructural metrics (MD). Furthest right column displays difference between association magnitudes of both models, as assessed by Williams' test.

eTable 8. Sensitivity analysis: health and lifestyle factors added as covariates into inflammation-white matter tract regressions (FA).

White matter tract FA	Side	(H0)		(H1)		(H2)		(H3)		(H4)		(H5)		(H6)		(H7)	
		Model 1		Fully-adjusted model		hypertension		CVD history		diabetes		BMI		alcohol		smoking	
		β	p(FDR)	β	p(FDR)	β	p(FDR)	β	p(FDR)	β	p(FDR)	β	p(FDR)	β	p(FDR)	β	p(FDR)
Corpus callosum	Genu	-0.119	0.008	-0.090	0.058	-0.117	0.010	-0.116	0.011	-0.124	0.006	-0.111	0.016	-0.119	0.008	-0.106	0.022
	Splenium	-0.078	0.077	-0.014	0.763	-0.072	0.101	-0.074	0.095	-0.079	0.076	-0.054	0.233	-0.078	0.077	-0.050	0.265
Arcuate fasciculus	Left	-0.108	0.014	-0.097	0.040	-0.100	0.023	-0.102	0.021	-0.112	0.012	-0.096	0.033	-0.108	0.014	-0.109	0.017
	Right	-0.150	0.001	-0.129	0.008	-0.143	0.002	-0.144	0.002	-0.152	0.001	-0.137	0.003	-0.150	0.001	-0.147	0.002
Anterior thalamic radiation	Left	-0.120	0.007	-0.101	0.033	-0.110	0.012	-0.117	0.008	-0.114	0.011	-0.108	0.018	-0.120	0.007	-0.124	0.007
	Right	-0.126	0.004	-0.066	0.153	-0.120	0.006	-0.121	0.006	-0.128	0.004	-0.095	0.032	-0.126	0.004	-0.105	0.020
Rostral cingulum	Left	-0.050	0.253	-0.029	0.536	-0.051	0.246	-0.048	0.278	-0.055	0.217	-0.031	0.486	-0.050	0.254	-0.046	0.312
	Right	-0.140	0.002	-0.108	0.023	-0.134	0.003	-0.142	0.002	-0.143	0.002	-0.128	0.005	-0.140	0.002	-0.120	0.009
Uncinate fasciculus	Left	-0.106	0.020	-0.068	0.161	-0.101	0.026	-0.101	0.027	-0.103	0.025	-0.091	0.051	-0.106	0.020	-0.097	0.038
	Right	-0.099	0.025	-0.058	0.222	-0.094	0.035	-0.091	0.040	-0.103	0.021	-0.077	0.091	-0.099	0.025	-0.088	0.055
Inferior longitudinal fasciculus	Left	-0.032	0.466	-0.010	0.837	-0.029	0.514	-0.027	0.541	-0.032	0.467	-0.025	0.569	-0.032	0.466	-0.022	0.620
	Right	-0.084	0.054	-0.048	0.296	-0.076	0.079	-0.078	0.075	-0.087	0.047	-0.067	0.131	-0.084	0.054	-0.072	0.110

Note: Inflammation-FA regression models with added health covariates entered individually. Standardised betas (p values) reported. bold typeface denotes $p < 0.05$ (FDR corrected). The following regressions are reported:

[H0] Brain health phenotype ~ DNAm CRP + age + sex

[H1] Brain health phenotype ~ DNAm CRP + age + sex + hypertension + CVD history + diabetes + BMI + alcohol + smoking

eTable 9. Sensitivity analysis: anti-inflammatory drug status, health and lifestyle factors added as covariates into inflammation-white matter tract regressions (MD).

White matter tract MD	Side	(H0)		(H1)		(H2)		(H3)		(H4)		(H5)		(H6)		(H7)	
		Model 1		Fully-adjusted model		hypertension		CVD history		diabetes		BMI		alcohol		smoking	
		β	p(FDR)	β	p(FDR)	β	p(FDR)	β	p(FDR)	β	p(FDR)	β	p(FDR)	β	p(FDR)	β	p(FDR)
Corpus callosum	Genu	0.060	0.180	0.077	0.105	0.064	0.154	0.064	0.157	0.062	0.168	0.083	0.070	0.060	0.182	0.054	0.245
	Splenium	0.079	0.074	0.058	0.217	0.076	0.088	0.085	0.056	0.080	0.074	0.080	0.076	0.079	0.075	0.055	0.223
Arcuate fasciculus	Left	0.105	0.016	0.104	0.027	0.098	0.025	0.101	0.022	0.102	0.021	0.112	0.012	0.105	0.016	0.099	0.028
	Right	0.111	0.015	0.106	0.029	0.107	0.018	0.112	0.014	0.111	0.015	0.115	0.013	0.110	0.015	0.098	0.036
Anterior thalamic radiation	Left	0.083	0.063	0.108	0.024	0.080	0.072	0.082	0.066	0.077	0.087	0.107	0.018	0.083	0.063	0.089	0.054
	Right	0.016	0.717	0.016	0.737	0.015	0.735	0.011	0.806	0.014	0.746	0.025	0.581	0.016	0.718	0.013	0.768
Rostral cingulum	Left	0.078	0.073	0.081	0.081	0.081	0.062	0.075	0.084	0.083	0.058	0.086	0.052	0.078	0.073	0.070	0.117
	Right	0.093	0.033	0.091	0.050	0.093	0.034	0.098	0.025	0.088	0.044	0.104	0.020	0.093	0.033	0.080	0.075
Uncinate fasciculus	Left	0.046	0.318	0.061	0.212	0.043	0.347	0.043	0.349	0.041	0.374	0.067	0.152	0.045	0.323	0.049	0.298
	Right	0.139	0.002	0.140	0.003	0.137	0.002	0.136	0.002	0.136	0.002	0.150	0.001	0.139	0.002	0.136	0.003
Inferior longitudinal fasciculus	Left	0.054	0.220	0.090	0.052	0.055	0.212	0.059	0.179	0.059	0.178	0.086	0.054	0.054	0.219	0.050	0.268
	Right	0.013	0.763	0.019	0.680	0.010	0.813	0.011	0.797	0.019	0.665	0.030	0.506	0.013	0.763	0.001	0.975

Note: Inflammation-MD regression models with added health covariates entered individually. Standardised betas (p values) reported. bold typeface denotes $p < 0.05$ (FDR corrected). The following regressions are reported:

[H0] Brain health phenotype ~ DNAm CRP + age + sex

[H1] Brain health phenotype ~ DNAm CRP + age + sex + hypertension + CVD history + diabetes + BMI + alcohol + smoking

eTable 10: Bivariate associations among study variables

Study variables	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)
(1) DNAm CRP	1.000																	
(2) serum CRP	0.288	1.000																
(3) BMI	0.189	0.292	1.000															
(4) hypertension	0.059	0.047	0.208	1.000														
(5) diabetes	0.121	0.027	0.162	0.123	1.000													
(6) CVD history	0.129	0.002	0.079	0.194	0.080	1.000												
(7) smoking	0.221	0.138	-0.066	0.023	-0.054	-0.028	1.000											
(8) alcohol consumption	0.241	0.060	0.014	0.048	-0.013	0.106	0.047	1.000										
(9) gf	-0.162	-0.092	-0.080	-0.082	-0.088	-0.062	-0.153	0.004	1.000									
(10) processing speed	-0.147	-0.083	-0.127	-0.090	-0.102	-0.065	-0.181	0.045	0.789	1.000								
(11) visuospatial ability	-0.072	-0.079	0.011	-0.074	-0.061	-0.056	-0.148	0.057	0.810	0.546	1.000							
(12) verbal memory	-0.116	-0.040	-0.025	0.005	-0.070	0.006	-0.085	0.013	0.586	0.382	0.418	1.000						
(13) TB	-0.249	-0.026	-0.117	-0.038	-0.104	-0.110	-0.164	-0.213	0.172	0.205	0.093	0.133	1.000					
(14) GM	-0.213	-0.023	-0.124	-0.100	-0.081	-0.049	-0.124	-0.122	0.134	0.147	0.099	0.099	0.741	1.000				
(15) NAWM	-0.182	-0.023	-0.092	-0.053	-0.070	-0.071	-0.169	-0.150	0.241	0.312	0.155	0.161	0.530	0.188	1.000			
(16) WMH	0.103	0.018	0.076	0.137	0.023	-0.005	0.099	0.021	-0.176	-0.230	-0.141	-0.100	0.006	-0.219	-0.585	1.000		
(17) gFA	-0.160	-0.054	-0.159	-0.146	0.002	-0.086	-0.094	-0.108	0.118	0.159	0.056	0.060	0.251	0.210	0.386	-0.370	1.000	
(18) gMD	0.126	0.024	-0.047	0.049	0.012	0.019	0.063	-0.009	-0.093	-0.148	-0.092	-0.124	-0.049	-0.105	-0.313	0.428	-0.480	1.000

Note. Pearson's r reported. TB: total brain volume, WMH: white matter hyperintensity volume, GM: grey matter volume; NAWM: normal appearing white matter volume. gf, general cognitive ability; gFA: general fractional anisotropy, gMD: general mean diffusivity; CVD: cardiovascular disease history

eTable 11. Results of single SEM mediation models assessing the relationship of brain structure with cognitive ability.

Mediator variable	Independent variable	Model	mediation (ab)			Total effect (c)			Direct effect (c')			Attenuation
			β	SE	p	β	SE	p	β	SE	p	
TB volume	serum CRP	model 1	-0.007	0.007	0.345	-0.099	0.038	0.009	-0.092	0.038	0.015	19.46%
		model 2	0.001	0.006	0.874	-0.061	0.040	0.127	-0.061	0.039	0.119	
	DNAm CRP	model 1	-0.031	0.011	0.005	-0.158	0.037	1.91E-05	-0.128	0.038	0.001	
		model 2	-0.018	0.008	0.051	-0.102	0.039	0.009	-0.083	0.039	0.032	
GM volume	serum CRP	model 1	-0.004	0.005	0.492	-0.099	0.038	0.010	-0.095	0.038	0.012	14.02%
		model 2	0.002	0.004	0.641	-0.060	0.040	0.128	-0.062	0.040	0.116	
	DNAm CRP	model 1	-0.022	0.010	0.025	-0.158	0.037	1.97E-05	-0.136	0.038	3.36E-04	
		model 2	-0.014	0.008	0.077	-0.101	0.039	0.009	-0.088	0.039	0.025	
NAWM volume	serum CRP	model 1	-0.009	0.010	0.377	-0.098	0.038	0.010	-0.089	0.037	0.017	21.00%
		model 2	0.001	0.009	0.886	-0.060	0.040	0.129	-0.062	0.039	0.114	
	DNAm CRP	model 1	-0.033	0.011	0.003	-0.159	0.037	1.85E-05	-0.125	0.037	0.001	
		model 2	-0.018	0.010	0.065	-0.102	0.039	0.008	-0.084	0.038	0.029	
WMH volume	serum CRP	model 1	-0.007	0.011	0.510	-0.098	0.038	0.010	-0.091	0.037	0.014	13.57%
		model 2	0.002	0.011	0.852	-0.060	0.040	0.131	-0.062	0.039	0.111	
	DNAm CRP	model 1	-0.021	0.011	0.060	-0.158	0.037	2.04E-05	-0.136	0.037	1.85E-04	
		model 2	-0.011	0.011	0.330	-0.101	0.039	0.009	-0.091	0.038	0.017	
gFA volume	serum CRP	model 1	-0.006	0.006	0.258	-0.099	0.038	0.009	-0.093	0.038	0.015	9.19%
		model 2	0.000	0.004	0.954	-0.061	0.040	0.126	-0.061	0.040	0.124	
	DNAm CRP	model 1	-0.015	0.008	0.079	-0.158	0.037	1.97E-05	-0.144	0.038	1.38E-04	
		model 2	-0.008	0.006	0.189	-0.101	0.039	0.009	-0.094	0.039	0.016	
gMD volume	serum CRP	model 1	-0.002	0.004	0.571	-0.098	0.038	0.010	-0.096	0.038	0.011	4.69%
		model 2	-0.002	0.003	0.551	-0.060	0.040	0.130	-0.058	0.040	0.143	
	DNAm CRP	model 1	-0.007	0.006	0.229	-0.158	0.037	2.04E-05	-0.151	0.037	5.87E-05	
		model 2	-0.007	0.006	0.265	-0.101	0.039	0.009	-0.094	0.039	0.016	

Note: Model 1 = Brain health variable ~ age + sex + inflammation;

Model 2 = Brain health variable ~ age + sex + inflammation + BMI + hypertension + smoking status + alcohol use + CVD history + diabetes

eTable 12. Results of multiple mediation models (TB, GM, NAWM, WMH, gFA, gMD) assessing the relationship of brain structure with cognitive ability (gf) and cognitive domains of visuospatial ability, processing speed and verbal memory

Outcome variable	Mediator variable	Independent variable	Model	mediation (ab)			Total effect (c)			Direct effect (c')			Attenuation
				β	SE	p	β	SE	p	β	SE	p	
g	Brain structure	serum CRP	model 1	-0.008	0.012	0.650	-0.099	0.038	0.021	-0.091	0.037	0.029	29.7%
			model 2	0.007	0.011	0.715	-0.060	0.040	0.282	-0.067	0.039	0.199	
		DNAm CRP	model 1	-0.047	0.015	0.004	-0.159	0.037	8.27E-05	-0.112	0.038	0.007	37.5%
			model 2	-0.023	0.013	0.167	-0.102	0.039	0.029	-0.079	0.039	0.108	
Visuospatial ability	Brain structure	serum CRP	model 1	-0.005	0.010	0.676	-0.081	0.038	0.054	-0.077	0.037	0.067	37.5%
			model 2	0.004	0.009	0.800	-0.071	0.039	0.166	-0.075	0.039	0.131	
		DNAm CRP	model 1	-0.036	0.013	0.013	-0.097	0.037	0.016	-0.061	0.038	0.144	41.0%
			model 2	-0.020	0.012	0.170	-0.051	0.038	0.308	-0.031	0.039	0.548	
Processing speed	Brain structure	serum CRP	model 1	-0.011	0.014	0.631	-0.086	0.038	0.048	-0.075	0.037	0.079	41.0%
			model 2	0.005	0.013	0.852	-0.029	0.039	0.640	-0.034	0.038	0.565	
		DNAm CRP	model 1	-0.058	0.016	0.001	-0.143	0.038	0.001	-0.084	0.038	0.044	33.3%
			model 2	-0.031	0.015	0.105	-0.066	0.040	0.197	-0.035	0.039	0.510	
Verbal memory	Brain structure	serum CRP	model 1	-0.002	0.009	0.830	-0.046	0.038	0.357	-0.044	0.038	0.377	33.3%
			model 2	0.003	0.009	0.873	-0.029	0.040	0.661	-0.032	0.040	0.620	
		DNAm CRP	model 1	-0.032	0.013	0.026	-0.096	0.038	0.026	-0.064	0.039	0.152	29.7%
			model 2	-0.022	0.012	0.141	-0.076	0.040	0.141	-0.054	0.041	0.327	

Results of multiple SEM mediation models (where TB, GM, NAWM, WMH, gFA, gMD are entered simultaneously) assessing the inter-relationship between inflammation, brain structure and cognitive measures. The final column ‘attenuation’ indicates the β -ratio (the proportion of inflammation’s effect on cognition that is explained by brain structural variable). For example, all brain structural variables accounted for 29.7% of the association between DNAm CRP and general cognitive ability, whereas the same brain structural variables accounted for 41% of the association between DNAm CRP and processing speed. Model 1 = Brain structure variable ~ age + sex + inflammation; Model 2 = Brain structure variable ~ age + sex + inflammation + BMI + hypertension + smoking status + alcohol use + CVD history + diabetes

eTable 13. Individual contributions to multiple mediation models (TB, GM, NAWM, WMH, gFA, gMD) assessing the relationship of brain structure with cognitive ability (gf) and cognitive domains of visuospatial ability, processing speed and verbal memory

Mediator variable	Independent variable	Model	gf mediation (ab)			visuospatial ability mediation (ab)			processing speed mediation (ab)			verbal memory mediation (ab)		
			β	SE	p	β	SE	p	β	SE	p	β	SE	p
			-0.008	0.012	0.650	-0.005	0.010	0.676	-0.011	0.014	0.631	-0.002	0.009	0.830
$\sum[\text{GM, NAWM, WMH, gFA, gMD}]$	serum CRP	model 1	0.007	0.011	0.715	0.004	0.009	0.800	0.005	0.013	0.852	0.003	0.009	0.873
		model 2	-0.047	0.015	0.004	-0.036	0.013	0.013	-0.058	0.016	0.001	-0.032	0.013	0.026
	DNAm CRP	model 1	-0.023	0.013	0.167	-0.020	0.012	0.170	-0.031	0.015	0.105	-0.022	0.012	0.141
		model 2	-0.002	0.003	0.512	-0.002	0.004	0.527	-0.002	0.003	0.521	-0.002	0.003	0.553
GM volume	serum CRP	model 1	0.001	0.003	0.659	0.002	0.003	0.632	0.001	0.002	0.657	0.001	0.003	0.628
		model 2	-0.016	0.009	0.091	-0.019	0.009	0.050	-0.015	0.009	0.102	-0.011	0.009	0.204
	DNAm CRP	model 1	-0.009	0.007	0.232	-0.012	0.007	0.120	-0.007	0.007	0.271	-0.007	0.007	0.347
		model 2	-0.008	0.009	0.391	-0.005	0.007	0.464	-0.009	0.011	0.393	-0.003	0.005	0.511
NAWM volume	serum CRP	model 1	0.001	0.008	0.881	0.002	0.006	0.794	0.001	0.010	0.896	0.001	0.004	0.763
		model 2	-0.030	0.012	0.012	-0.024	0.011	0.023	-0.037	0.013	0.006	-0.017	0.009	0.064
	DNAm CRP	model 1	-0.014	0.009	0.095	-0.011	0.007	0.121	-0.018	0.011	0.087	-0.007	0.006	0.210
		model 2	0.002	0.003	0.580	0.001	0.002	0.639	0.002	0.003	0.579	0.002	0.003	0.554
WMH volume	serum CRP	model 1	0.004	0.004	0.350	0.002	0.003	0.562	0.003	0.004	0.379	0.004	0.004	0.349
		model 2	-0.002	0.006	0.795	4.68E-04	0.006	0.937	-0.003	0.006	0.585	0.001	0.006	0.890
	DNAm CRP	model 1	-0.003	0.004	0.448	-0.001	0.002	0.722	-0.003	0.004	0.465	-0.002	0.003	0.507
		model 2	-4.41E-04	0.003	0.883	0.003	0.004	0.410	-0.001	0.003	0.826	0.004	0.004	0.377
gFA volume	serum CRP	model 1	1.58E-06	1.39E-04	0.991	-1.38E-04	0.003	0.959	0.000	0.000	0.979	0.000	0.003	0.968
		model 2	-0.001	0.009	0.866	0.010	0.009	0.245	-0.001	0.008	0.889	0.008	0.009	0.340
	DNAm CRP	model 1	-2.68E-04	0.006	0.965	0.007	0.007	0.306	0.000	0.006	0.935	0.005	0.006	0.458
		model 2	0.001	0.002	0.712	-0.001	0.002	0.623	0.000	0.002	0.827	-0.003	0.005	0.542
gMD volume	serum CRP	model 1	0.001	0.002	0.720	-0.001	0.002	0.663	-0.001	0.002	0.690	-0.003	0.005	0.473
		model 2	0.002	0.006	0.789	-0.004	0.006	0.490	-0.003	0.006	0.671	-0.012	0.008	0.110
	DNAm CRP	model 1	0.003	0.007	0.668	-0.003	0.007	0.646	-0.003	0.007	0.615	-0.011	0.008	0.172

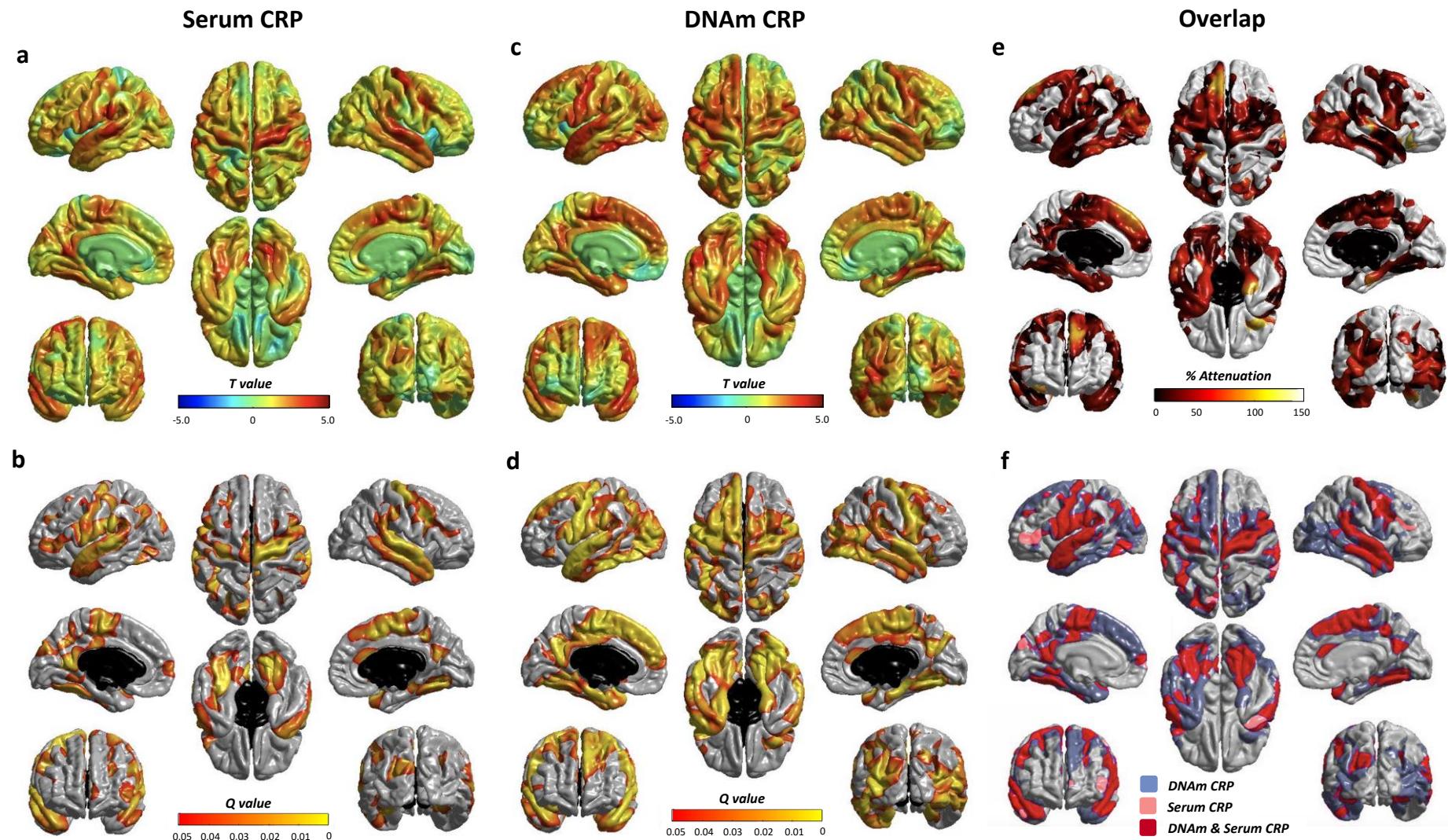
Note: Model 1 = Brain health variable ~ age + sex + inflammation;

Model 2 = Brain health variable ~ age + sex + inflammation + BMI + hypertension + smoking status + alcohol use + CVD history + diabetes

eTable 14: Tract loadings for general factors of white matter fractional anisotropy and mean diffusivity

<i>White matter tract</i>	<i>PC1(FA)</i>	<i>PC1 (MD)</i>
Genu of corpus callosum	0.647	0.646
Splenium of corpus callosum	0.490	0.308
Left arcuate fasciculus	0.722	0.735
Right arcuate fasciculus	0.69	0.767
Left anterior thalamic radiation	0.65	0.72
Right anterior thalamic radiation	0.634	0.645
Left rostral cingulum	0.55	0.648
Right rostral cingulum	0.573	0.752
Left uncinate fasciculus	0.637	0.665
Right uncinate fasciculus	0.669	0.744
Left Inferior longitudinal fasciculus	0.494	0.41
Right Inferior longitudinal fasciculus	0.477	0.371
Proportion of variance	0.370	0.405

Supplementary eFigure 1



eFigure 1. DNAm CRP shows stronger and more widespread associations with regional brain cortical thickness than serum CRP. Regional cortical thickness regressed against serum CRP (i-ii) and DNAm CRP (iii-iv). Colours denote the magnitude (T-maps; top) and significance (Q values; bottom) of the negative associations between inflammation and brain cortical thickness. Panel (v) shows the percentage attenuation for the significant associations between DNAm-CRP and cortical thickness when also controlling for serum CRP. Conjunction plot (vi) shows the spatial extent of independent contributions and overlap (red) in cortical loci that exhibit FDR-corrected unique associations with simultaneously-modelled serum (pink) and epigenetic (blue) inflammation measures; results are corrected for sex, age and ICV.

Supplementary eFigure 2.

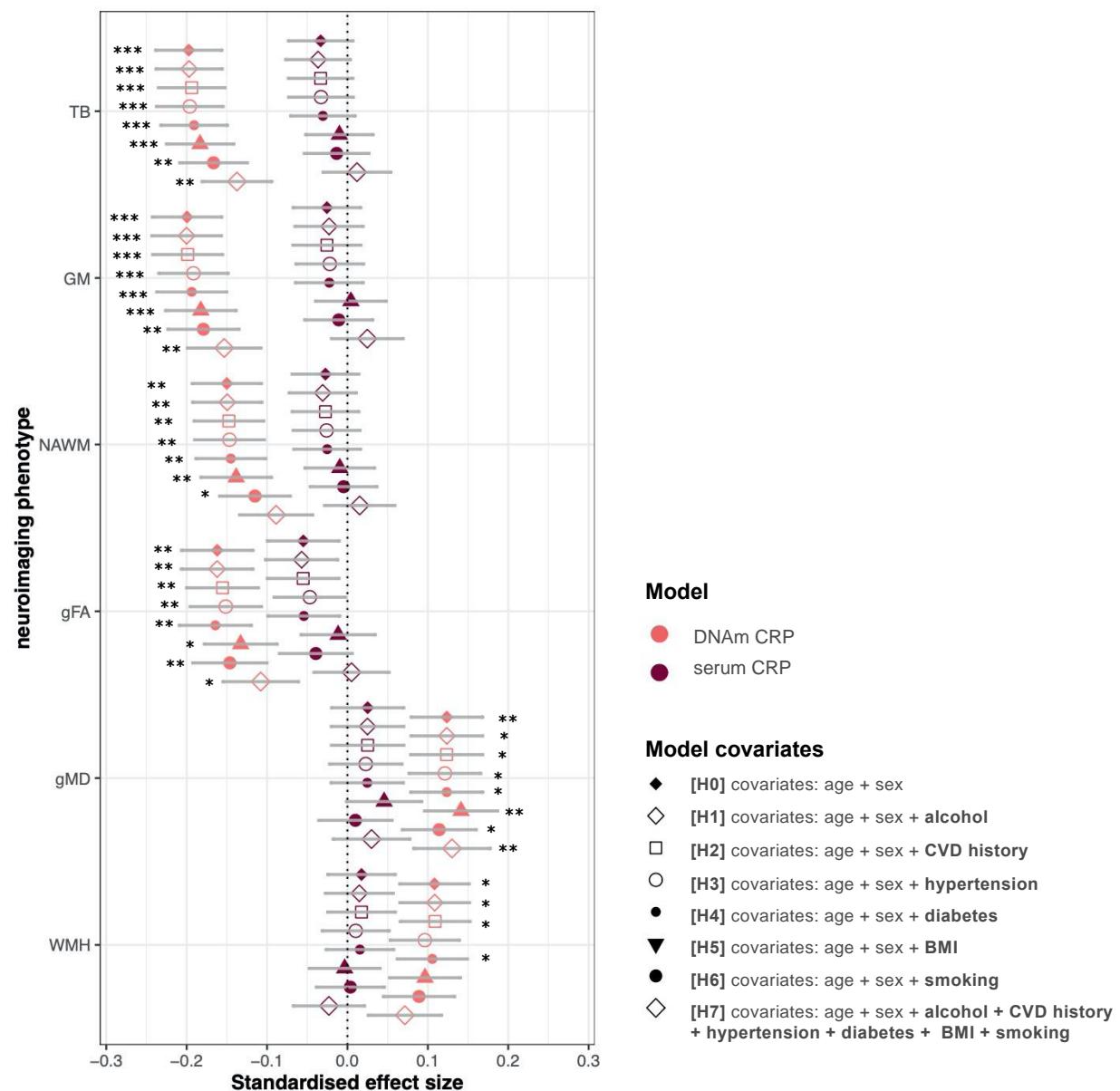


Fig. e-2. Associations between serum and epigenetic CRP measures and brain structure and the impact of lifestyle covariates on associations; shapes show standardised regression coefficients for different models (corresponding with table e-6), error bars show standard errors. TB: total brain, GM: grey matter, NAWM: normal-appearing white matter, WMH: white matter hyperintensity, gf, general cognitive ability; gFA: general fractional anisotropy, gMD: general mean diffusivity.

Supplementary eFigure 3.

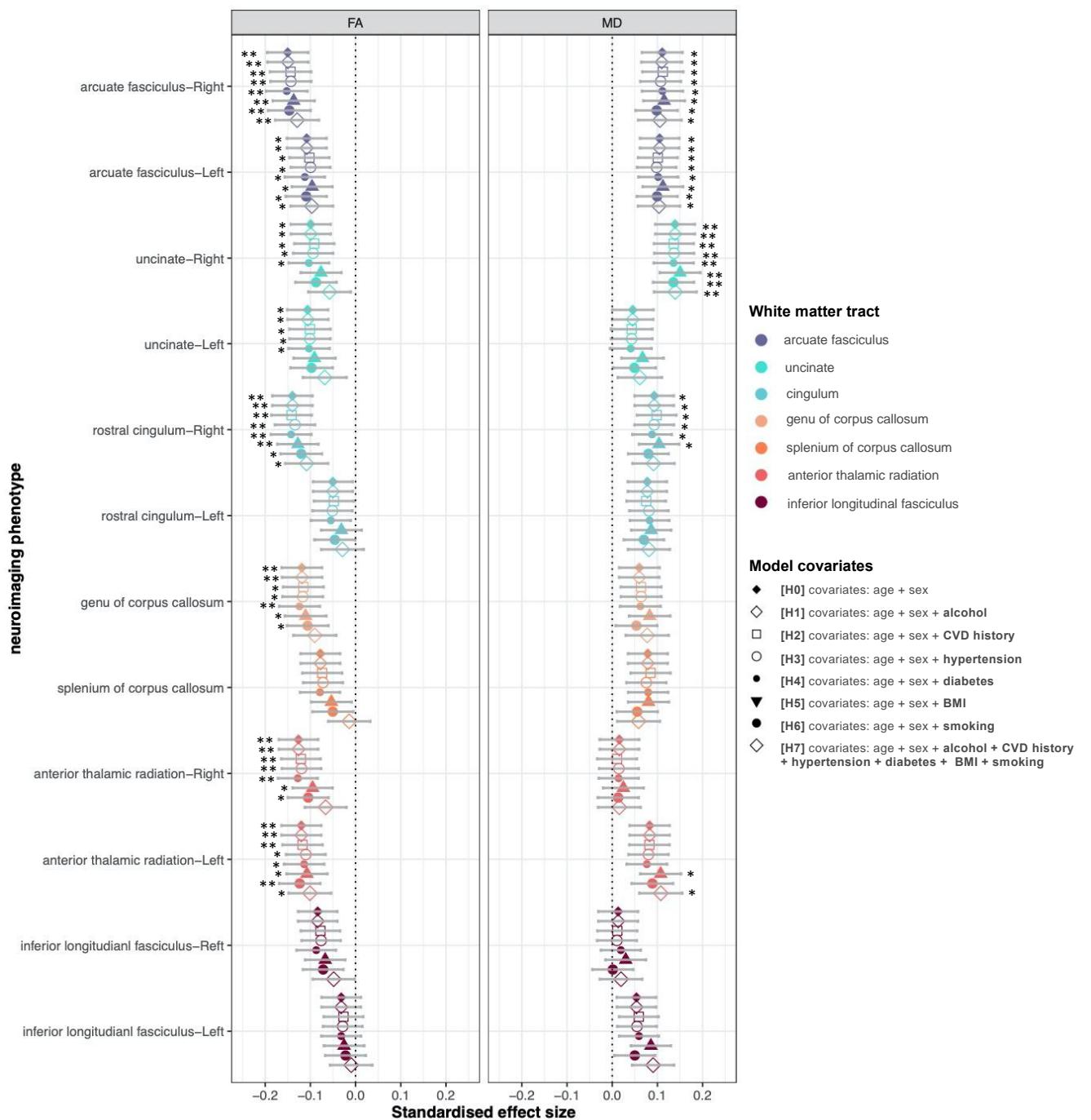
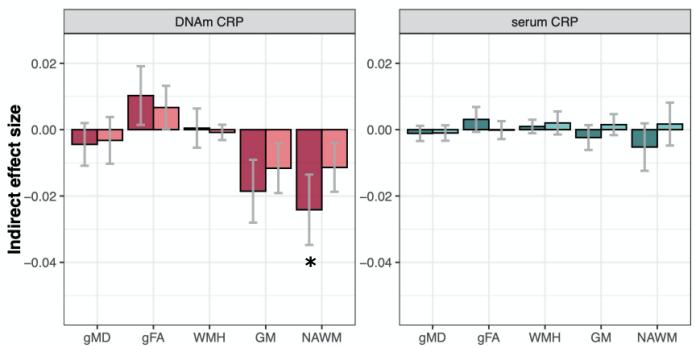
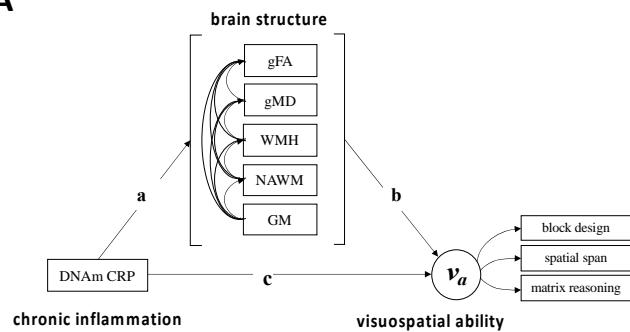


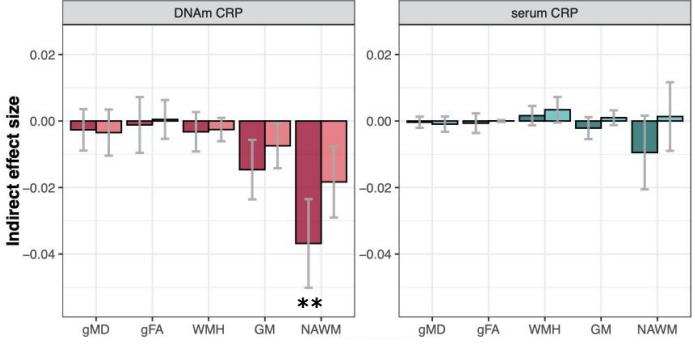
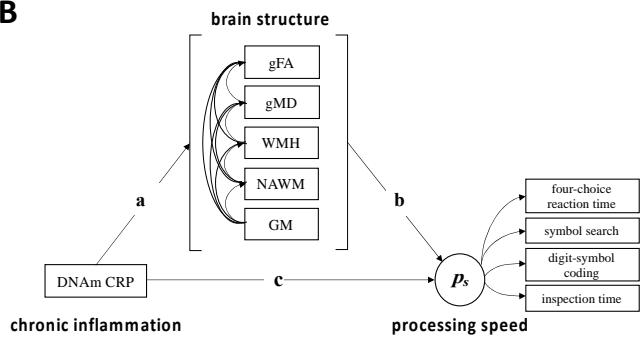
Fig. e-3. Associations between serum and epigenetic CRP measures and WM tract FA and MD and the impact of lifestyle covariates on associations; shapes show standardised regression coefficients for different models (corresponding with eTable 9 and eTable 10), error bars show standard errors.

Supplementary eFigure 4.

A



B



C

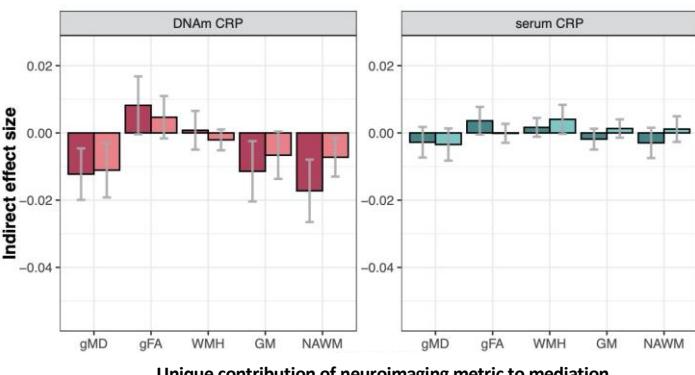
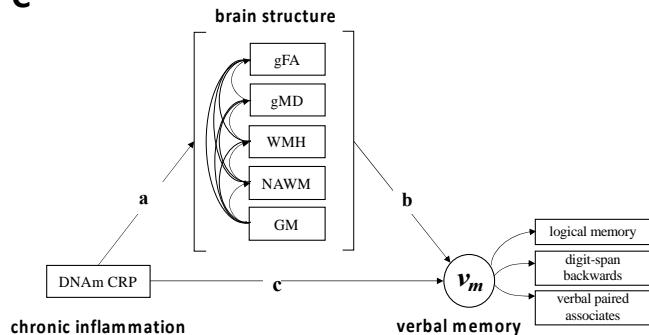


Fig e.4. Multiple mediator models of the association of inflammation with individual cognitive domains

Left displays structural equation model path diagrams; right displays mediation of individual MRI metrics (indirect effect size on y axis) and standard error bars. Light bars show model 1 (includes covariates age and sex), dark bars show model 2 which contains additional health covariates (age + sex + BMI + hypertension + smoking status + alcohol use + CVD history + diabetes). Asterisks denote FDR $p < 0.05$. TB: total brain, GM: grey matter, NAWM: normal-appearing white matter, WMH: white matter hyperintensity, gf, general cognitive ability; gFA: general fractional anisotropy, gMD: general mean diffusivity; $n = 521$

Supplementary eFigure 5.

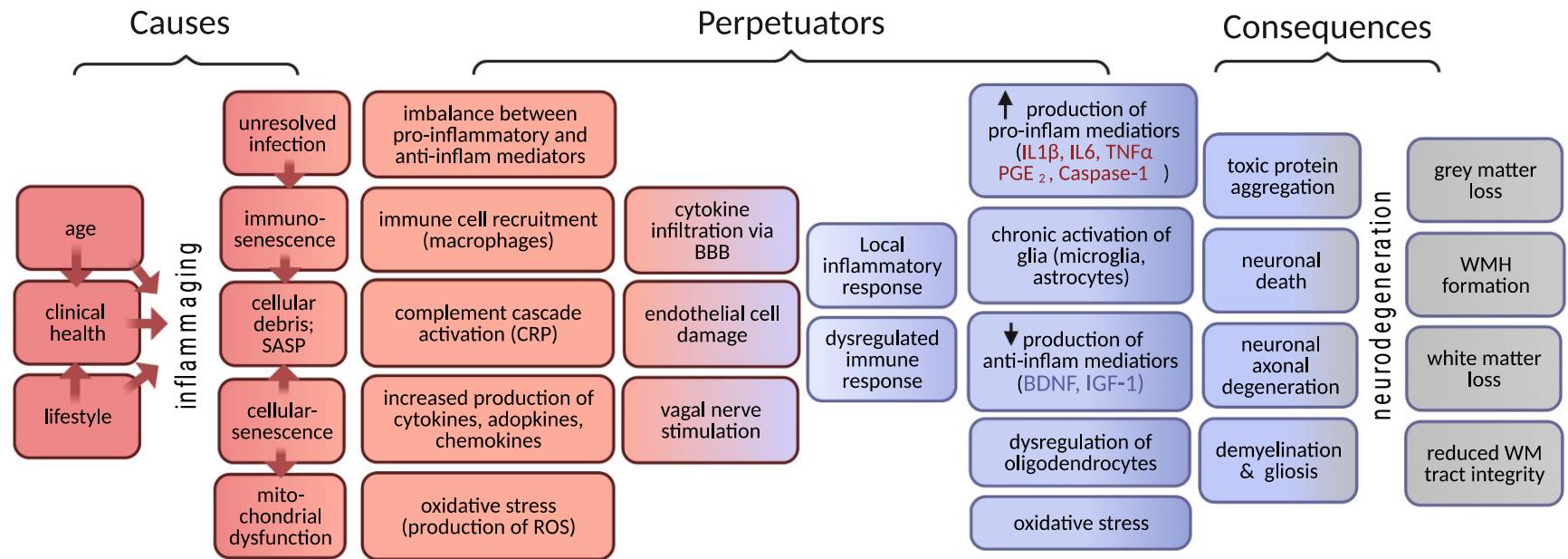


Fig e.5. Mechanisms of neurodegeneration via increased systemic chronic inflammation

Suggested mechanisms by which the causes of inflammaging (immunosenescence, lifestyle, clinical health) and related consequences may drive brain health (structural and cognitive) outcomes.

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