

# An epigenetic proxy of chronic inflammation outperforms serum levels as a biomarker of brain ageing

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## Abstract

2 Low-level chronic inflammation increases with age and is associated with cognitive decline. DNA  
3 methylation (DNAm) levels may provide more stable reflections of cumulative inflammatory burden  
4 than traditional serum approaches. Using structural and diffusion MRI data from 521 individuals aged  
5 73, we demonstrate that a DNAm proxy of C-Reactive Protein (CRP) shows significantly (on average  
6 6.4-fold) stronger associations with brain structural outcomes than serum CRP. We additionally find  
7 that DNAm CRP has an inverse association with global and domain-specific (speed, visuospatial and  
8 memory) cognitive functioning, and that brain structure partially mediates this CRP-cognitive  
9 association (up to 29.4%), dependent on lifestyle and health factors. These data support the hypothesis  
10 that chronic systemic inflammation may contribute to neurodegenerative brain changes which underlie  
11 differences in cognitive ability in later life. DNA methylation-based predictors could be used as proxies  
12 for chronic inflammatory status.

13

**Main text:**

14 Low-level, systemic chronic inflammation has emerged as a hallmark and potential driver for  
15 individual differences in brain ageing, with numerous animal and human studies showing links  
16 between sub-acute chronic inflammation and poor brain health<sup>1–7</sup>. While acute inflammation is a  
17 healthy, short-term reaction to tissue damage or infection, low-level chronic inflammation is  
18 characterised by an ongoing, heightened activity of the immune system that damages cells and tissues.  
19 Incidence of peripheral chronic inflammation appears to increase as we age and is strongly associated  
20 with age-related diseases including dementia and cardiovascular disease<sup>8</sup>. A striking feature of  
21 cognitive ageing at the population level is its wide heterogeneity, with some individuals experiencing  
22 more rapid or severe cognitive decline than others<sup>9</sup> (Figure 1); the determinants of such differences in  
23 age-related cognitive decline are not fully understood, but the shift to a progressive, chronic  
24 inflammatory state in older-age – termed ‘inflammaging’<sup>10</sup> – may be a key contributor to inter-  
25 individual differences in cognitive ageing.

26 However, while chronic inflammation has been independently linked to various neurodegenerative  
27 phenotypes<sup>11–14</sup>, the relationship between low level systemic chronic inflammation and cognitive  
28 decline in healthy ageing (i.e. in the absence of neurodegenerative disease) is less clearly defined.  
29 Studies using serum inflammatory measures in non-clinical groups show mixed positive, negative and  
30 null associations with respect to cognitive outcomes<sup>2,15–21</sup>, and have not yet clarified the magnitude  
31 and regional extent of brain structural associations<sup>3–6,22,23</sup>. Limitations of previous studies include  
32 disparity in methodology (different imaging metrics and cognitive measures), small sample sizes  
33 (typically, n <100, although a recent study looked at chronic inflammation in association with  
34 cognitive and neuroimaging measures in over 2,000 participants<sup>23</sup>) and a lack of screening for acutely  
35 elevated inflammation levels suggestive of infection<sup>24</sup>. Variation in participant health is a likely  
36 source of such heterogeneity between study cohorts, as different lifestyle factors are known to  
37 increase susceptibility to chronic inflammation (e.g. smoking, obesity, and alcohol consumption) and  
38 the related health consequences of inflammation-driven damage (including metabolic syndrome, type-

39 2 diabetes, cardiovascular disease and neurodegenerative pathology) may serve to perpetuate a  
40 chronic inflammatory state<sup>8,25</sup>. Moreover, while many studies have investigated the relationship  
41 between chronic inflammation and brain health outcomes, few consider all three variables  
42 (inflammation, cognitive ability, neuroimaging) concurrently<sup>4</sup>.

43 Perhaps the largest shortcoming of previous work, however, is how low-level chronic inflammation is  
44 measured. As there are no standard biomarkers for low-level chronic inflammation, to date many  
45 studies have relied upon canonical blood biomarkers of acute inflammation such as C-Reactive  
46 Protein (CRP) to index chronic inflammatory states. A caveat of this methodology is inferring  
47 baseline inflammation levels from highly phasic protein levels, which are subject to swift and rapid  
48 concentration changes in blood plasma. Serum CRP levels, for example, can increase up to 1,000-fold  
49 in response to infection and vary from 1 µg/mL to 500 µg/mL within 24-72h of tissue injury<sup>26</sup>. Thus,  
50 the properties which make CRP a good measure of acute infection also introduce significant noise  
51 when looking at chronic inflammation associations at the epidemiological level (see Figure 1a). Few  
52 studies take repeated measures of serum CRP or attempt to control for this variation when  
53 investigating relationships with CRP and brain health phenotypes; where this is collected, longitudinal  
54 stability in CRP profiles is low, with large intra-subject variability<sup>27</sup>.

55 Recent advances in the use of epigenetics to investigate long-term health outcomes<sup>21,28,29</sup> may present  
56 a solution to the challenge of reliably recording average inflammation levels. DNA methylation  
57 (DNAm) is an epigenetic mechanism where the addition of a methyl group to a cytosine-phosphate-  
58 guanine (CpG) nucleotide base pairing regulates gene activity<sup>30</sup>. DNA methylation (DNAm) profiles  
59 are increasingly recognised as biomarkers of disease status and historical archives of environmental  
60 exposures<sup>31-33</sup> and differential DNAm profiles have been identified in inflammatory diseases<sup>34,35</sup> and  
61 inflammation-related disease outcomes<sup>36</sup>. In contrast to genetic variation, DNAm levels vary across  
62 the lifecourse<sup>37</sup> and can be affected by both genes and the environment<sup>38</sup>; measuring DNAm profiles  
63 may therefore allow us to tease apart which aspects of lifestyle contribute to chronic inflammation. In  
64 addition, while DNAm levels are dynamic, their short term variability is relatively stable<sup>39</sup>, and they

65 may reflect longer-term immune function<sup>40</sup>. In the same cohort as in the present study, a DNAm  
66 proxy of CRP exhibited greater longitudinal stability and stronger associations with a single global  
67 measure of cognitive functioning than blood based CRP levels<sup>21,41</sup>. Given this temporal stability, a  
68 DNAm signature of inflammation could represent a cumulative, aggregate measure of chronic  
69 inflammation analogous to the HbA1c test used to index average blood sugar levels for diabetics<sup>21</sup>.  
70 We tested this suspected increase in signal:noise conferred by DNAm vs serum CRP on a large  
71 number of global and regional measures of brain grey and white matter health to interrogate how  
72 inflammation might contribute to cognitive ageing differences.

73 To date no study has investigated a DNAm measure of CRP relation to structural brain health  
74 outcomes. Here we present a detailed comparison of association magnitudes between a serum and  
75 DNAm measure of CRP in relation to multiple global and regional structural brain imaging measures,  
76 and to various aspects of cognitive function and lifestyle in a well-characterised community-dwelling  
77 sample of healthy adults in older age. We hypothesised that our epigenetic score of CRP, created from  
78 an out of sample epigenome-wide association study (EWAS), would show significantly stronger  
79 associations with brain health than serum levels across a range of detailed neuroimaging and cognitive  
80 measures. We additionally investigate whether the association of inflammation with cognitive ability  
81 is mediated via alterations to brain structure and how lifestyle factors affect this relationship.

82

## Results

83 **DNAm CRP is associated with global and regional brain volume**

84 We studied 521 older adults (aged ~ 73 years; see Supplementary Table 1) and looked at epigenetic vs  
85 serum inflammation associations across a range of cognitive, neuroimaging and lifestyle measures  
86 (Supplementary Table 2). To index chronic inflammation, an epigenetic measure of CRP (DNAm  
87 CRP) was assembled for each participant: methylation beta values for 7 CpG sites shown to have the  
88 strongest association with serum CRP levels were derived, multiplied by their standardised regression  
89 weights (taken from the largest meta-EWAS of CRP to date<sup>34</sup>, where CpGs were replicated across a  
90 range of cohorts: European, n = 8,863, and African-American, n=4,111), and summed to generate a  
91 single DNAm CRP score per subject (CpG weights presented in Supplementary Table 5). We found  
92 that higher inflammatory burden, indexed by DNAm CRP scores, associated with poor cognitive and  
93 neuroimaging brain health outcomes (Supplementary Table 2). DNAm CRP exhibited significantly  
94 larger associations with brain structural MRI metrics (including global grey and white matter atrophy,  
95 poorer white matter microstructure and increased white matter hyperintensity burden) than serum  
96 CRP associations.

97 Figure 2A illustrates these effect size differences for global neuroimaging measures, which were  
98 larger by 6.4-fold, on average, for DNAm CRP vs serum CRP associations. These DNAm CRP-  
99 associated brain structural changes were independent of anti-inflammatory drug-use and vascular risk  
100 factors (Supplementary Table 5). Participants with a higher inflammatory burden on average had  
101 greater overall brain atrophy, with higher DNAm CRP associating with lower total brain volume ( $\beta =$   
102  $-0.197$ ,  $p_{FDR} = 8.42 \times 10^{-6}$ ), grey matter volume ( $\beta = -0.200$ ,  $p_{FDR} = 1.66 \times 10^{-5}$ ) and white matter  
103 volume ( $\beta = -0.150$ ,  $p_{FDR} = 0.001$ ).

104 After examining global brain structural alterations, we looked at specific regional cortical brain  
105 associations with higher inflammation levels. We found regional heterogeneity in the patterning of  
106 associations between CRP measures and cortical metrics: atrophy in frontal, anterior lateral and  
107 medial temporal lobes were associated with higher DNAm CRP (Figure 2B); inflammation

108 associations with brain cortical thickness are presented in the supplementary document  
109 (Supplementary Figure 2). Overall, these results emphasise that the DNAm-CRP score associates with  
110 lower cortical volume of specific brain regions (lateral and medial temporal regions of the brain),  
111 which show overlap with those of serum CRP and unique variance (Figure 2B-vi), with DNAm CRP  
112 reflecting atrophy above and beyond the serum CRP score.

113 **DNAm CRP is associated with white matter microstructure in specific white matter tracts**

114 Next, we investigated whether higher DNAm CRP was related to lower white matter microstructure  
115 based on global and regional diffusion MRI (dMRI) measures by looking at inflammation  
116 associations with white matter tract fractional anisotropy (FA; the directional coherence of water  
117 molecule diffusion) and mean diffusivity (MD; the magnitude of water molecule diffusion). While  
118 serum CRP-dMRI associations were null in all cases (all  $p_{FDR} > 0.089$ ) (Supplementary Tables 7 and  
119 8), higher DNAm CRP score predicted overall lower general fractional anisotropy (gFA)  
120 ( $\beta = -0.162$ ,  $p_{FDR} = 6.94 \times 10^{-4}$ ) and higher general mean diffusivity (gMD) ( $\beta = 0.124$ ,  $p_{FDR} = 0.010$ ).  
121 For specific white matter tracts, the strongest associations were seen for the arcuate fasciculus and  
122 uncinate fasciculus, with lower FA and higher MD with higher DNAm CRP (see Figure 3;  
123 Supplementary Tables 7-8). For both global and regional measures across grey and white matter,  
124 accounting for anti-inflammatory drug status and health and lifestyle covariates did not substantially  
125 alter the magnitude or significance of these associations (Supplementary Tables 9 and 10).

126 **Brain structure partly mediates the association of DNAm CRP with cognitive ability**

127 As higher DNAm CRP levels were associated with lower cognitive performance both here  
128 (Supplementary Table 2) and previously<sup>21</sup>, we quantified the degree to which brain structural  
129 differences contribute to the inflammation-cognition association, and which facets show the strongest  
130 unique contributions to this relationship. We used a structural equation modelling (SEM) framework  
131 to simultaneously characterise the associations among CRP, brain and cognitive metrics, and also  
132 specifically test the hypothesis that brain structure partially and significantly mediates associations  
133 between measures of CRP and cognitive ability. Bivariate associations between all variables

134 (inflammation, brain structure, cognitive ability and lifestyle measures) are provided in the  
135 Supplementary document (Supplementary Table 8). While total brain (TB) volume, grey matter (GM)  
136 volume, normal appearing white matter (NAWM) volume and white matter hyperintensity (WMH)  
137 volume all emerged as significant mediators in single SEM models (percentage attenuation 10-21%;  
138 Supplementary Table 12), multiple mediator models were used to test the degree to which each global  
139 MRI metric contributed uniquely to mediation of the same association (Figure 4d). Here, the sum total  
140 of MRI measures significantly mediated the association between DNAm CRP and general cognitive  
141 ability ( $\beta = -0.047$ , pFDR = 0.002; percentage attenuation 29.4%). The unique contributions to this  
142 variance (Figure 4d and Supplementary Table 13) were largest for NAWM volume ( $\beta = -0.029$ , pFDR  
143 = 0.012) indicating that the loss of white matter integrity in particular may contribute to  
144 inflammation-associated differences in cognitive functioning in older age. Finally, with the addition  
145 of lifestyle and health covariates to our models, no aspect of brain structure remained a significant  
146 mediator of the associations between DNAm CRP and general cognitive ability (see Figure 4,  
147 Supplementary Tables 12 and 13).

148

## Discussion

149 In summary, this is the first time that an epigenetic score of CRP has been shown to associate with  
150 differences in structural brain measures. We discovered that DNAm CRP shows consistently stronger  
151 associations with brain structure than serum CRP (on average, 6.4 fold greater), that these  
152 associations are not regionally homogeneous across the brain's cortex, and that specific aspects of  
153 brain structure partly mediate (up to 29.4%) associations between an epigenetic signature of CRP and  
154 cognitive functioning, with lifestyle factors significantly attenuating this relationship.

155 We found regional heterogeneity in the patterning of associations between CRP measures and cortical  
156 metrics, indicating differential regional vulnerability to chronic inflammation. Atrophy in frontal,  
157 anterior lateral and medial temporal lobes were associated with increased DNAm CRP. The regional  
158 overlap between DNAm CRP and serum CRP brain atrophy was strikingly consistent for both cortical  
159 thickness and cortical volume, with higher DNAm CRP relating to a thinner cortex and lower volume  
160 across a more widespread range of regions, but also identical regions to that of serum CRP.

161 Consistently, previous studies report structural changes associated with inflammatory markers in the  
162 temporal and frontal cortices<sup>22,42</sup>. Differential patterns of pro-inflammatory receptor distribution in  
163 brain vasculature and tissue are thought to contribute to both local and global brain atrophy<sup>43</sup>, and  
164 increased receptor expression may underlie why some brain regions are more vulnerable to  
165 inflammation than others. For example, in post-mortem Alzheimer's disease (AD) patients, pro-  
166 inflammatory cytokine receptor density and expression were upregulated in regions of  
167 neurodegeneration, including the medial frontal and temporal cortices<sup>44</sup>. Divergent inflammatory  
168 markers are also associated with ischemic and haemorrhagic stroke phenotypes, indicating that  
169 differing inflammatory pathways and their impact on regional brain vasculature may underlie  
170 subtypes of stroke<sup>45</sup>. Although the participants in this study were free of neurodegenerative  
171 phenotypes such as stroke or AD, it is possible that sub-incident pathology (undetected lacunar stroke,  
172 prodromal dementia) could account for our inflammation-atrophy associations. Moreover, a study on

173 subjects free of history stroke or AD found that CRP was significantly related to progression of  
174 carotid atherosclerosis, and that CRP had differential effects in different beds of the arterial brain  
175 supply<sup>46</sup>. Overall, it appears that raised levels of inflammatory mediators contribute to localised brain  
176 atrophy via their differential and detrimental effects on cerebrovasculature.

177 Our findings support the hypothesis that chronic systemic inflammation may contribute to changes in  
178 brain structure which underlie differences in cognitive ability in later life (see Supplementary Figure 1  
179 for suggested mechanisms). As normal appearing white matter volume emerged as the largest  
180 contributor to the mediation of the association between DNAm CRP and a global measure of  
181 cognitive ability, we suggest that the brain's white matter may be particularly vulnerable to the  
182 damaging impact of chronic inflammation, and that loss of white matter integrity may drive  
183 inflammation-associated accelerated cognitive ageing. Relatedly, several studies have found  
184 associations between reduced white matter volume and raised inflammatory mediators both in healthy  
185 cohorts<sup>5,19,47</sup> and those with chronic inflammatory conditions<sup>48</sup>. Possible mechanistic causes for this  
186 matter-specific brain atrophy emerge from post-mortem studies looking at microglia deposition: in  
187 AD brain samples, microglia activation is specifically increased in white matter relative to grey<sup>49,50</sup>.  
188 Chronically elevated inflammation levels in the periphery can cause microglia to shift from a state of  
189 comparative quiescence to one of chronic activation<sup>51</sup>, resulting in an enhanced inflammatory  
190 response promoting neuronal cell death and subsequent cognitive decline. Moreover, microglia can  
191 deteriorate the blood-brain barrier (BBB) from inside the brain, resulting in further infiltration of  
192 inflammatory mediators from the systemic circulation (see Supplementary Figure 1)<sup>52</sup>. White matter is  
193 supplied by the perforating arterioles and there is evidence that people with greater WMH burden  
194 exhibit subtly increased BBB leakage, and in turn, that BBB leakage is associated with worse  
195 cognition at a one year follow-up<sup>53</sup>. In a study on patients with systemic inflammatory arthropathies,  
196 elevated inflammatory mediators were associated with increased imaging signs of small vessel disease  
197 including perivascular spaces and WMH<sup>54</sup>. The areas of brain loss that were particularly associated  
198 with the epigenetic CRP here (temporal cortices) are also areas where others have shown increased  
199 BBB leakage in persons at risk of AD<sup>53,55</sup>. It therefore seems that damage to the BBB is a possible

200 pathway through which sustained levels of inflammation in the periphery results in  
201 neurodegeneration. In addition, increased endothelial inflammation markers have been reported in  
202 subjects with increased WMHs<sup>56</sup>, providing more evidence for neurovascular mediated  
203 neuroinflammation and subsequent downstream white matter loss.

204 In line with this, we found that higher DNAm CRP is related to ostensibly poorer white matter  
205 microstructure (lower FA and higher MD). In particular, the white matter tracts of arcuate fasciculus  
206 and uncinate fasciculus showed the most consistent significant relationships with DNAm CRP levels  
207 (across both FA and MD), alongside significantly lower FA in the anterior thalamic radiation, which  
208 is susceptible to the effects vascular risk and cognitive ageing<sup>57</sup>. Similar cohorts also show links  
209 between white matter integrity and chronic inflammation: a faster decline in serum CRP levels was  
210 related to greater white matter tract health, with declines in inflammation over six years predicting FA  
211 in various white matter tracts<sup>47</sup>. Similarly, higher midlife CRP levels predicted reduced white matter  
212 integrity in later life<sup>5</sup>. As with discrete regional cortical thinning, our results indicate that loss of white  
213 matter integrity occurs at both regional and global levels, indicating that individual white matter tracts  
214 may have differential vulnerability to the effects of chronic inflammation. Stronger microstructural  
215 associations with DNAm CRP than serum CRP point towards potential utility in monitoring chronic  
216 inflammation in AD or small vessel disease populations via epigenetic markers to characterise and  
217 quantify inflammatory status.

218 Given that all mediations via brain structure were significantly attenuated by the addition of lifestyle  
219 factors into our models, it is clear that various exposures and predisposing health attributes influence  
220 the association between chronic inflammation and brain health. These included factors that showed  
221 significant associations with higher inflammation levels (BMI, diabetes, smoking, alcohol  
222 consumption and hypertension) in this study (see Supplementary Table 2) and others<sup>8,58</sup>. Many of the  
223 lifestyle attributes associated with chronic inflammation (and probable perpetrators of it) are at least  
224 partly modifiable, yet studies that have successfully targeted these risk factors and shown  
225 corresponding reductions in inflammation levels are scarce<sup>8</sup>. Variation in serum levels may be

226 confounding this relationship, and our data suggest that the DNAm-based predictor may act as a  
227 quantifiable archive of the longitudinal effects of these exposures, and other unaccounted for health  
228 and genetic profiles, that serum CRP levels fail to capture.

229 The strengths of the present work include the large sample size, array of multi-modal data, narrow age  
230 range and ethnic homogeneity of our cohort; the concurrently-collected data were optimal for  
231 interrogating the relationship between chronic inflammation and cognition from neuroimaging,  
232 cognitive, epigenetic and lifestyle angles. Compared to recent EWAS-neuroimaging research, this  
233 study is exceptionally well-powered with 521 participants after exclusions<sup>29</sup>. The narrow age-range  
234 and ethnic homogeneity are simultaneously limitations of this study, as they restrict the degree to  
235 which our findings can be related to other populations and limit our scope to identify inflammation-  
236 brain health associations at other times of life. Equally, given the cross-sectional nature of this study,  
237 we are unlikely to capture the effect of more age-related changes in inflammatory profile and  
238 cognitive decline. Observed cognitive and brain structural alterations could be independently related  
239 to a genetic-predisposition unaccounted for by our health and lifestyle covariates, such as metabolic-  
240 syndrome or more niche vascular vulnerability. Although we endeavoured to remove participants with  
241 cognition-related pathology (stroke, AD, Parkinson's disease and MCI), these were screened via self-  
242 reported diagnoses and we may be missing undiagnosed or subclinical incident neurodegenerative  
243 pathology. While it is exciting to consider that DNAm levels could provide more accurate reflections  
244 of chronic inflammatory status, more work is required to determine the direction and strength of this  
245 association with brain health phenotypes. It is possible that chronic inflammation is not a cause, rather  
246 a marker of, or even a response to, unrelated neurodegenerative phenotypes that can lead to changes  
247 in cognitive ability. To disentangle directionality of effects, there is demand for studies that collect  
248 repeated measures of DNAm, cognitive and neuroimaging data over time. A few cohorts, including  
249 LBC1936, are suited to this type of longitudinal study as highlighted in a recent systematic review<sup>59</sup>;  
250 to further strengthen causal inference or in cases where no such data is available, a two-sample  
251 Mendelian Randomisation approach may be best suited.

252 These findings have clinical implications, for example, relating DNAm proxies of inflammatory  
253 exposure to MRI patterns in neonates could shed light on the role of chronic inflammation and brain  
254 structure in preterm infants, where chronic inflammation is of known brain health consequence but  
255 disentangling maternal vs infant exposure is difficult<sup>60</sup>. Future studies should consider examining a  
256 wider range of DNAm inflammatory markers (DNAm levels of interleukins, prostaglandins,  
257 neurotrophins); looking at DNAm inflammatory markers in younger participants (where there is likely  
258 greater variation in baseline inflammation levels); and looking at DNAm inflammatory markers in  
259 specific brain pathology cases (e.g. multiple sclerosis patients).

260 In conclusion, these findings support the hypothesis that chronic systemic inflammation may  
261 contribute to neurodegenerative brain changes which underlie differences in cognitive ability in later  
262 life. Previous studies exploring this relationship may underestimate the brain and cognitive sequelae  
263 of chronic inflammation by relying on single measurements of phasic serum proteins. By utilising an  
264 epigenetic inflammation measure, which integrates information from multiple immune-related CpG  
265 sites, we may provide a more reliable measure of chronic inflammation and thus a more  
266 comprehensive overview of the consequences of chronic inflammation on brain structure and  
267 function. Reliable monitoring of inflammatory exposure could enable clinicians to review the efficacy  
268 of drug and lifestyle interventions to attenuate inflammation levels with a view to improving cognitive  
269 outcomes.

270

## Materials and Methods

271 **Participants**

272 Data are drawn from the Lothian Birth Cohort 1936 (LBC1936). The cohort is composed of 1,091  
273 individuals, born in 1936 and re-contacted around 60 years later (mean age:  $69.6 \pm 0.8$  years). Most  
274 took part in the Scottish Mental Survey 1947 at age 11. Detailed cognitive, genetic, epigenetic, health  
275 and lifestyle data was collected at this first wave, and also three years later, supplemented with a  
276 detailed structural brain imaging protocol (see below). Participants were free of neurodegenerative  
277 diagnoses at baseline and, as the study's focus is on healthy cognitive ageing, were excluded if they  
278 had a self-reported history of stroke (n=55), Parkinson's (n= 5), or dementia (n=2) or had an MMSE <  
279 24, indicating mild cognitive impairment (n=7). We additionally excluded participants with serum  
280 CRP level  $>10\text{mg/L}$  (n = 32), suggestive of acute infection or illness at the time of blood draw<sup>24</sup>.  
281 After exclusions, a total of 680 participants had DNAm CRP data at age 73 years, 521 of whom also  
282 provided brain MRI data. We used the maximum available sample size in all analyses. All variables  
283 described in this study were collected at Wave 2.

284 **Brain imaging data**

285 Structural and diffusion tensor (DTI) MRI acquisition and processing in LBC1936 were performed  
286 according to an open-access protocol<sup>61</sup>. A 1.5 T GE Signa HDx clinical scanner (General Electric,  
287 Milwaukee, WI, USA) was used to collect structural T1-, T2-, T2\*-, and fluid attenuated inversion  
288 recovery-weighted images. Total brain (TB), grey matter (GM), normal-appearing white matter  
289 (NAWM) and white matter hyperintensity (WMH) volumes were segmented using a semi-automated  
290 multi-spectral technique<sup>62</sup>. Local processing and QC of cortical reconstruction and segmentation was  
291 performed using FreeSurfer v5.1 on T1-weighted volumes. Following visual inspection of the outputs  
292 (to check for aberrant surfaces and tissue segmentation failures, which were removed from analysis)  
293 these were registered to the fsaverage surface. White matter connectivity data – measures of fractional  
294 anisotropy (FA) and mean diffusivity (MD) – were created and segmented using the  
295 BEDPOSTX/ProbTrackX algorithm in FSL (<https://fsl.fmrib.ox.ac.uk>) and Tractor

296 (<https://www.tractor-mri.org.uk>). Using probabilistic neighbourhood tractography (PNT), tract-  
297 average white matter FA and MD were derived as the average of all voxels contained within the  
298 resultant tract maps (genu of corpus callosum; splenium of corpus callosum; arcuate fasciculus;  
299 anterior thalamic radiation; rostral cingulum; uncinate fasciculus; inferior longitudinal fasciculus), as  
300 described previously<sup>63,64</sup>. A general factor of FA ( $g_{FA}$ ) and MD ( $g_{MD}$ ) was derived for each participant  
301 from the first un-rotated principal component of a principal components analysis (PCA) on twelve of  
302 the white matter tracts FA and MD values; participants with up to 2 missing values from specific  
303 tracts had data replaced with the mean value for that tract. These general factors reflect common  
304 microstructural properties across main white matter pathways and capture the common variance in  
305 white matter integrity. Details of individual test loadings are provided in the supplementary document  
306 (Supplementary Table 3).

307 **C-Reactive Protein data**

308 Serum CRP was measured from whole-blood samples using a high sensitivity assay (enzyme-linked  
309 immunosorbent assay; R&D Systems, Oxford, UK)<sup>54</sup>.

310 **DNA methylation preparation and DNAm CRP score**

311 Genome-wide DNA methylation was measured in blood samples using the Illumina Human  
312 MethylationEPIC BeadChip at the Edinburgh Clinical Research Facility Genetics Core; details of this  
313 profiling has been outlined<sup>65</sup>. The DNAm CRP score was calculated for each participant as described  
314 previously<sup>66</sup>, briefly, a DNAm CRP score was assembled for each participant in Wave 2 of LBC1936;  
315 this was created by means of a weighted composite score, based on a discovery meta-analysis (9  
316 cohorts, n = 8,863) and a replication meta-analysis (4 cohorts, n = 4111) of CRP-EWAS studies<sup>34</sup>.  
317 Methylation beta values were derived for the 7 CpG sites shown to have the strongest association with  
318 serum CRP levels, and then multiplied by their standardised regression weights (taken from the meta-  
319 EWAS of CRP;<sup>34</sup>) and added together. Given that all regression weights from the EWAS were  
320 negative, a higher DNAm CRP score (i.e closer to 0) corresponds to a higher inflammatory profile.  
321 Relative weights for the 7 CpGs are included in the supplementary document (Supplementary Table  
322 5).

323 **Cognitive ability data**

324 A general fluid-type cognitive ability score (gf) was derived from the first un-rotated principal  
325 component of a PCA of relevant cognitive tests from the Wechsler Adult Intelligence Scale-Third  
326 Edition (WAIS-III<sup>UK</sup>)<sup>67</sup>. Relevant cognitive tests and individual weightings of gf and the latent  
327 variables of processing speed, visuospatial ability and verbal memory can be found in Supplementary  
328 Table 4.

329 **Lifestyle variables**

330 Lifestyle variables included body mass index (BMI; kg/m<sup>2</sup>) alongside variables relating to self-  
331 reported health and disease history: cardiovascular disease history (CVD); hypertension; diabetes;  
332 smoking status (coded as current smoker [1] versus ex/non-smoker [0]) and current alcohol use  
333 (alcohol units per week).

334 **Volumetric brain associations with inflammation**

335 Linear regression models were used to identify the proportion of phenotypic variance explained by  
336 DNA CRP and to determine whether this was independent of the serum CRP signal for each  
337 neuroimaging, cognitive and lifestyle phenotype. Logistic regressions were conducted for self-  
338 reported disease history variables as these had binary outcomes (disease/no disease). The phenotypic  
339 measure was the dependent variable, and the serum CRP or DNA CRP score was the independent  
340 variable of interest. Differences between association magnitudes (serum CRP vs epigenetic CRP  
341 associations) were assessed using the Williams' test for dependent groups with overlapping  
342 correlations (cocor.indep.groups.overlaps) as implemented in the cocor R package (<http://cran.r-project.org/web/packages/cocor/cocor.pdf>).  
343

344 **Regional brain analyses**

345 Localized associations between DNA CRP score and vertex-wise cortical volume, area and  
346 thickness were performed using linear regression, controlling for age, sex, and ICV. We used the  
347 SurfStat MATLAB toolbox (<http://www.math.mcgill.ca/keith/surf stat>) for Matrix Laboratory R2012a  
348 (The MathWorks, Inc., Natick, MA, USA). The resulting statistical maps (*t*-maps) were corrected for

349 multiple comparisons using FDR with a q-value of 0.05 across all 327,684 vertices on the cortical  
350 surface. Negative associations with CRP measures (e.g. lower volume with higher inflammation)  
351 were represented by the hot end of the colour spectrum. Following exclusions based on a history of  
352 stroke, dementia or an MMSE < 24, n = 521 had complete vertex-wise MRI, epigenetic and  
353 phenotypic data. White matter tract-specific associations with CRP measures were investigated using  
354 regression models adjusted for age and sex as described previously for global volumetric measures  
355 (TB, GM, NAWM, WMH).

### 356 **Specific white matter tract associations with inflammation**

357 As with volumetric brain-inflammation associations, regressions were run with regional FA and MD  
358 values of left and right projections of the individual white matter tracts (genu of corpus callosum;  
359 splenium of corpus callosum; arcuate fasciculus; anterior thalamic radiation; rostral cingulum;  
360 uncinate fasciculus; inferior longitudinal fasciculus) as dependent variables.

### 361 **Sensitivity analyses**

362 We sought to investigate whether anti-inflammatory drug status had an influence on our models. In a  
363 sensitivity analysis, anti-inflammatory drug status (collected at baseline and coded as a dichotomous  
364 variable: on medication = 1; not on medication = 0) was included as a covariates alongside age and  
365 sex. Similarly, we included lifestyle and health covariates separately in models (alongside age and  
366 sex) to determine whether individual aspects of health and lifestyle had an impact on the association  
367 of inflammation with brain-health phenotypes.

### 368 **Mediation analyses**

369 We ran mediation analyses in a structural equation modelling (SEM) framework using the R ‘lavaan’  
370 package (<https://cran.r-project.org/web/packages/lavaan/lavaan.pdf>). This simultaneously  
371 characterised associations among CRP, brain and cognitive metrics, and also specifically tested the  
372 hypothesis that brain structure would partly and significantly mediate associations between measures  
373 of CRP and cognitive ability. Both single and multiple mediator models were specified (see Figure  
374 4a-b as example). Single mediator models provided information on the proportion of CRP-cognitive

375 associations attributable to individual neuroimaging metrics. By contrast, in Multiple mediator  
376 models, brain structural variables were entered simultaneously as covarying mediators. This allowed  
377 us to quantify the proportion of variance in CRP-cognitive associations uniquely explained by each  
378 facet of brain structure (GM, NAWM, WMH, gFA, gMD). The primary estimates of interest in this  
379 study are the degree of change (mediation) in the direct path ( $c$  to  $c'$ ) between inflammation measures  
380 (DNAm CRP or serum CRP) and cognitive ability when the indirect path from inflammation to  
381 cognitive ability via brain structure ( $a \times b$ ) is included. A significant mediation of the  $c$  path (to  $c'$ ) is  
382 denoted by the statistical significance of this indirect effect. Bootstrapping was used calculate the  
383 standard errors. Multiple comparisons were corrected for by FDR correction. These mediations were  
384 re-run when accounting for self-reported health variables as covariates. In Model 1 age and sex were  
385 covariates; in Model 2, they were age, sex, BMI, hypertension, diabetes, smoking status and alcohol  
386 use. Model fit was evaluated based on root mean squared error approximation (RMSEA), the  
387 comparative fit index (CFI), the standardized root mean square residual (SRMR) and the Tucker–  
388 Lewis index (TLI). We considered a model an acceptable fit when it respected the following  
389 thresholds: RMSEA  $\leq 0.05$ ; SRMR  $\leq 0.06$ ; CFI  $\geq 0.97$ ; TLI  $\geq 0.95$  as recommended by<sup>68</sup>.

## 390 Statistical Analyses

391 Statistical analyses were performed in R version 3.6.1 (<https://www.r-project.org>). Alpha was 0.05 for  
392 all analyses and results were corrected for multiple comparisons using the false discovery rate (FDR)  
393 using the ‘p.adjust’ function in the ‘stats’ package in R. Standardized coefficients are reported  
394 throughout to facilitate comparison of associations. Serum measures of CRP were log-transformed to  
395 correct a positively skewed distribution. When conducting analysis for brain structural associations, to  
396 control for the confounding effect of head size, all global MRI volumetric measures (TB, GM, NAWM,  
397 WMH) were corrected for intracranial volume (ICV) and expressed as a ratio of ICV. Pairwise bivariate  
398 associations were assessed between markers of inflammation, neuroimaging and lifestyle covariates  
399 using Pearson correlation.



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### **Author contributions:**

E.L.S.C., S.R.C. and R.E.M. designed the analysis. E.L.S.C. conducted the analysis and drafted the work. A.J.S. prepared the DNA methylation data. S.M.M. provided the illustration in Figure 3B. S.E.H., S.M.M., M.V.H., M.A.H., M.E.B., J.M.W., I.J.D., and S.R.C. collected or analysed the brain MRI and/or cognitive data. All authors critically revised the work and have approved the submitted version. Data Availability

### **Data availability:**

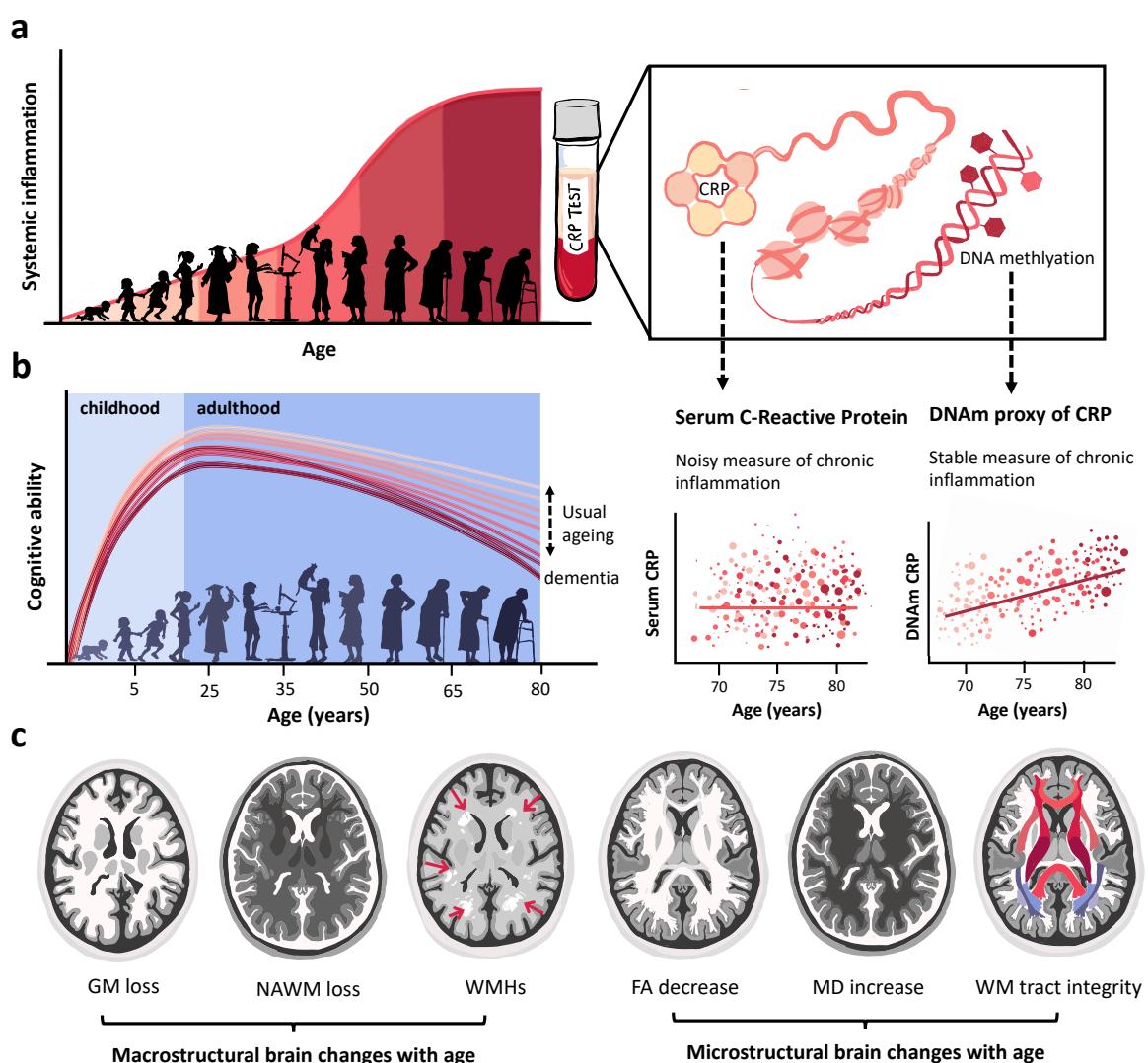
The data analysed in this study is not publicly available as it contains data that could compromise participant consent and confidentiality, but can be requested via a data access request to the Lothian Birth Cohorts research group.

### **Materials and correspondence:**

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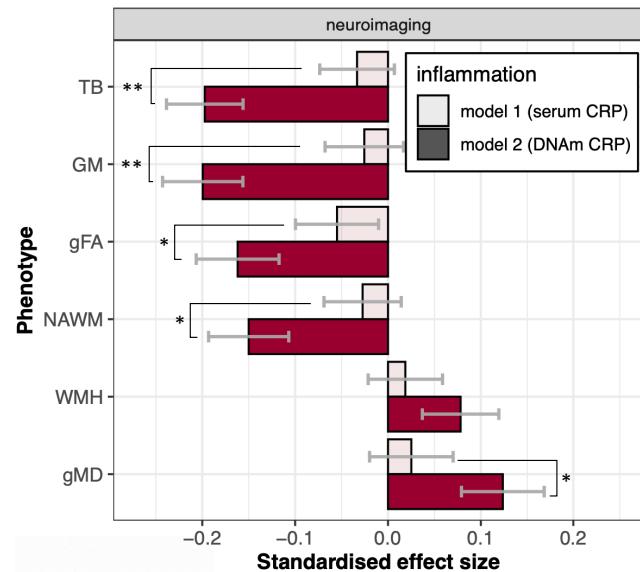
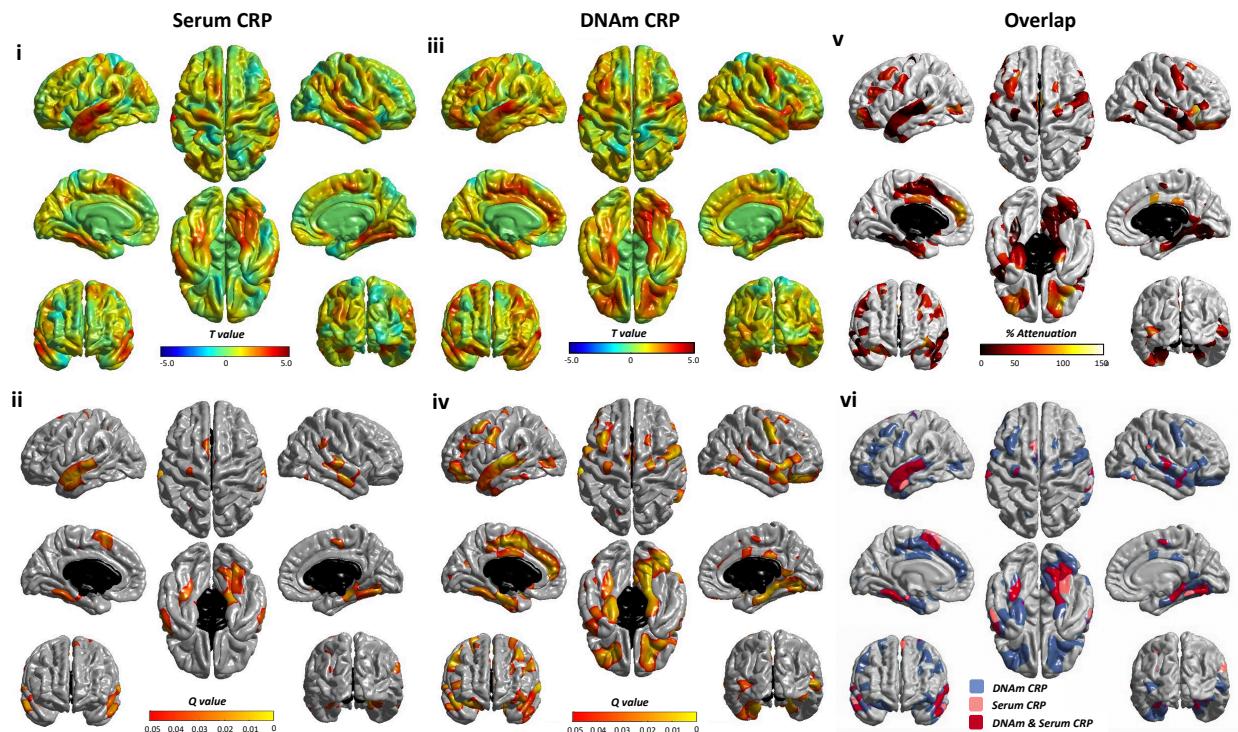
## Figures and Legends:

**Figure 1**



**Fig. 1. Chronic inflammation increases with age and may contribute to variance in cognitive ability and brain structure**

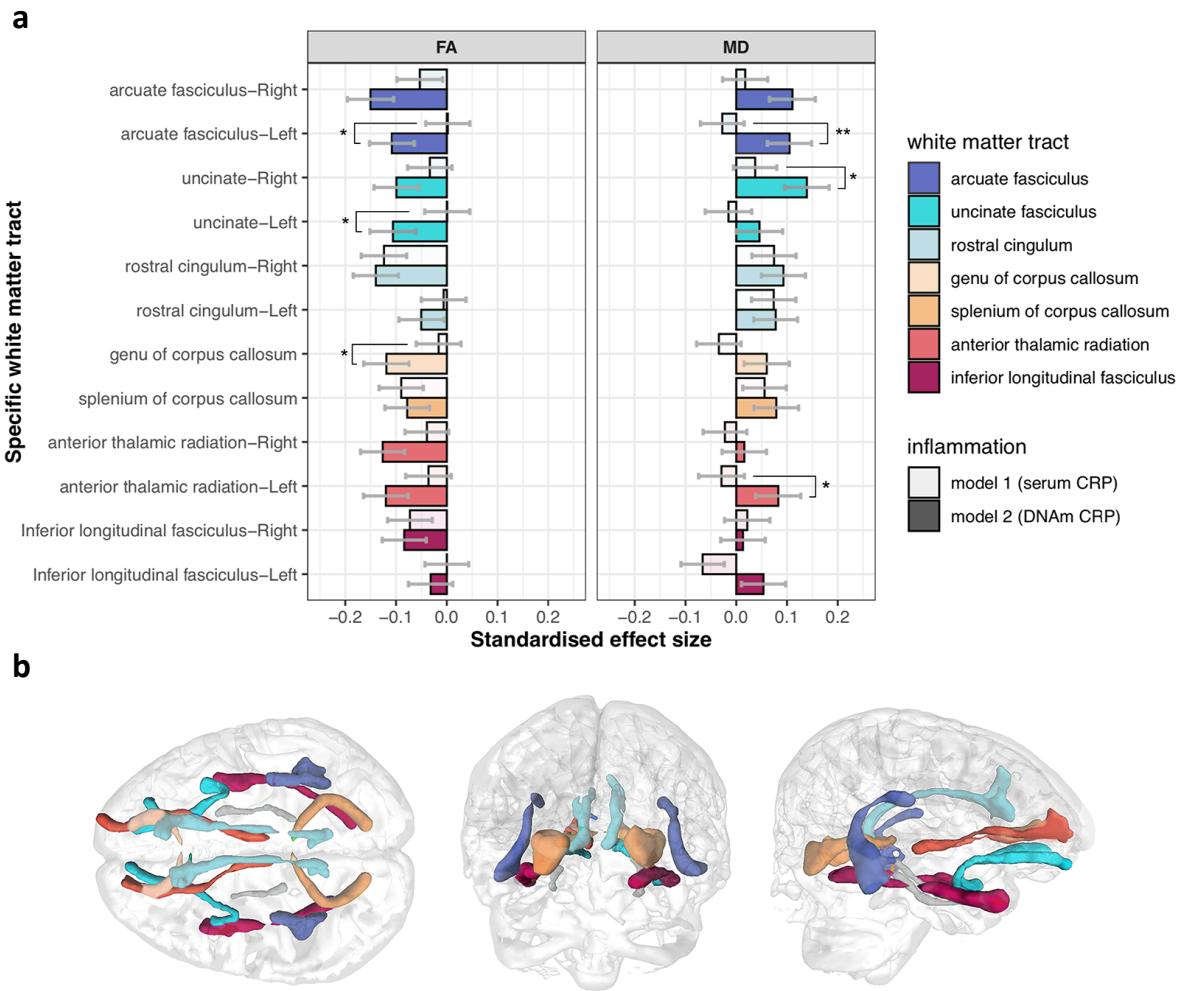
(a) Schematic demonstrating how chronic inflammation increases with age (left panel) and can be indexed by inflammatory proteins taken from a blood sample (right panel), such as serum levels and DNA methylation (DNAm) proxies of C-Reactive Protein. Graphs below reflect trajectories of respective inflammation scores over age, as outlined in<sup>21</sup>. (b) Life-span curves for cognitive ability, demonstrating that there is considerable inter-individual heterogeneity in rate and timing of cognitive decline, with some people on more accelerated cognitive ageing trajectories than others<sup>9</sup>. (c) From left to right, schematic diagram displays structural and diffusion MRI (T1, T2, T2-FLAIR weighted, DTI) correlates of cognitive decline which include alterations in brain macrostructure, such as atrophy in grey matter (GM), normal appearing white matter (NAWM) and increased presence of white matter hyperintensities (WMH; indicated by red arrows). Diffusion tensor imaging (DTI) can reveal alterations in measures of brain microstructure, such as global changes in fractional anisotropy (FA) and mean diffusivity (MD); loss of individual white matter tract integrity can be inferred from probabilistic neighbourhood tractography (PNT) to extract white matter (WM) tracts of interest from DTI data.

**Figure 2****a Global brain associations****b Regional brain associations****Fig. 2. DNAm CRP shows stronger and more widespread associations with global and regional brain structure than serum CRP**

(a) Associations between CRP measures and brain structure ( $n = 521$ ); bars show standardised regression coefficients, error bars show standard errors. Asterisks indicate significant differences (\* $P < 0.05$ , \*\* $P < 0.01$ ; williams' test) between serum CRP and DNAm CRP coefficients, which were 6.4-fold larger, on average, for DNAm CRP.

(b) Regional cortical volume regressed against serum CRP (i-ii) and DNAm CRP (iii-iv)  $n = 521$ . Colours denote the magnitude (T-maps; top) and significance (Q values; bottom) of the negative associations between inflammation and brain cortical volume. Panel (v) shows the percentage attenuation for the significant associations between DNAm-CRP and cortical volume 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. TB: total brain, GM: grey matter, NAWM: normal-appearing white matter, WMH: white matter hyperintensity, g<sub>f</sub>: general cognitive ability; gFA: general fractional anisotropy, gMD: general mean diffusivity.

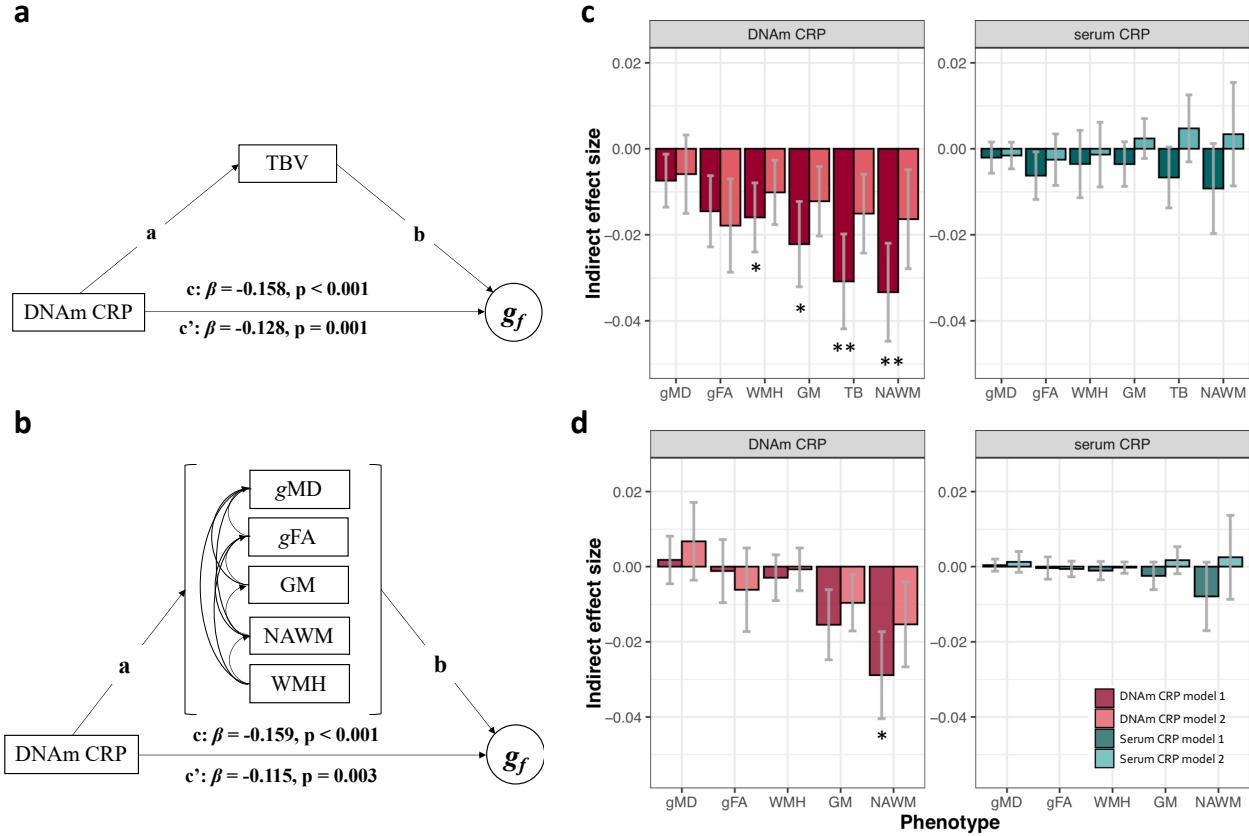
**Figure 3**



**Fig.3. DNAm CRP is associated with white matter microstructure in specific white matter tracts**

**(a)** Standardised regression coefficients for associations between white matter tract-averaged fractional anisotropy (FA; left), and mean diffusivity (MD; right). Bars show standardised coefficients and standard errors. Asterisks indicate where associations are significantly larger for DNAm than for serum (\* $P<0.05$ , \*\* $P<0.01$ ; williams' test). (arcuate fasciculus n =513; anterior thalamic radiation n = 516; rostral cingulum n=507, genu of corpus callosum n =497; splenium of corpus callosum n =509; inferior longitudinal fasciculus n =516) **(b)** illustration of the respective white matter tracts measured using probabilistic neighbourhood tractography in one LBC1936 study participant.

**Figure 4**



**Fig.4. Brain structure partly mediates the association of DNAm CRP with cognitive ability**

Top panel (a-c) displays single mediator models, bottom panel (b-d) displays multiple mediator models.

(a) Model 1 structural equation model path diagrams showing that in model 1 the association between DNAm CRP and general cognitive ability (path c) was significantly partially mediated by total brain volume (path ab = -0.031, p = 0.005), attenuating the c path by 19.5% (path c'), and (b) 29.4% by multiple MRI variables (ab = -0.047, p = 0.002) (c) Single mediator models indirect effect size and standard error bars. (d) Multiple mediator models indirect effect size 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 denotes 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.