Associations of circulating C-reactive proteins, APOE ε4, and brain markers for Alzheimer’s disease in healthy samples across the lifespan

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ABSTRACT

The apolipoprotein E gene ε4 allele (APOE ε4) and higher circulating level of C-reactive protein (CRP) have been extensively investigated as risk factors for Alzheimer’s disease (AD). Paradoxically, APOE ε4 has been associated with lower levels of blood CRP in middle-aged and older populations. However, few studies have investigated this intriguing relation and its impact on neurological markers for AD in younger ages, nor across the whole lifespan. Here, we examine associations of blood CRP levels, APOE ε4, and biomarkers for AD in a cognitively healthy lifespan cohort (N up to 749; 20–81 years of age) and replicate the findings in UK Biobank (N = 304 322; 37–72 years of age), the developmental ABCD study (N = 10 283; 9–11 years of age), and a middle-aged sample (N = 339; 40–65 years of age). Hippocampal volume, brain amyloid-β (Aβ) plaque levels, cerebrospinal fluid (CSF) levels of Aβ and tau species, and neurofilament protein light chain (NFL) were used as AD biomarkers in subsamples. In addition, we examined the genetic contribution to the variation of CRP levels over different CRP ranges using polygenic scores for CRP (PGS-CRP). Our results show APOE ε4 consistently associates with low blood CRP levels across all age groups (p < 0.05). Strikingly, both ε4 and PGS-CRP associated mainly with blood CRP levels within the low range (<5mg/L). We then show both APOE ε4 and high CRP levels associate with smaller hippocampus volumes across the lifespan (p < 0.025). APOE ε4 was associated with high Aβ plaque levels in the brain (FDR-corrected p = 8.69x10^{-4}), low levels of CSF Aβ42 (FDR-corrected p = 6.9x10^{-2}), and lower ratios of Aβ42 to Aβ40 (FDR-corrected p = 5.08x10^{-5}). Blood CRP levels were weakly correlated with higher ratio of CSF Aβ42 to Aβ40 (p = 0.03, FDR-corrected p = 0.4). APOE ε4 did not correlate with blood concentrations of another 9 inflammatory cytokines, and none of these cytokines correlated with AD biomarkers.

Conclusion: The inverse correlation between APOE ε4 and blood CRP levels existed before any pathological AD biomarker was observed, and only in the low CRP level range. Thus, we suggest to investigate whether APOE ε4 can confer risk by being associated with a lower inflammatory response to daily exposures, possibly leading to greater accumulation of low-grade inflammatory stress throughout life. A lifespan perspective is needed to understand this relationship concerning risk of developing AD.
1. Introduction

Late onset Alzheimer’s disease (AD) is the major cause of dementia, but our understanding of its aetiology is incomplete (Scheltens et al., 2021). Clinical manifestation of AD typically occurs after 60–65 years of age, and includes declined cognitive performance and brain atrophy (Burns and Iliffe, 2009), whereas the pre-symptomatic phase of AD could start 15–20 years before the clinical diagnosis. The most popular hypothesis for the development of AD – the amyloid beta (Aβ) cascade hypothesis (Beyreuther and Masters, 1991; Selkoe and Hardy, 2016) – suggests the disease is initiated by extracellular aggregation of Aβ plaques, which in turn causatively accumulation of intracellular neurofi brillary tangles composed of hyperphosphorylated tau proteins, and subsequently induces neuroinflammation in the brain. Ultimately, the cascade leads to widespread neuronal and synaptic dysfunction, brain atrophy, and cognitive impairment. Whereas the Aβ cascade hypothesis explains early-onset AD to a certain extent, it appears to play a smaller role in the more common late-onset form of AD. Recent genome-wide association studies (GWAS) for late-onset AD have reported up to 30 variants showing very weak associations to AD development. However, most of these variants show very weak associations with AD (Scheltens et al., 2021).

The apolipoprotein E gene (APOE) ε4 allele is the strongest genetic risk factor established for late onset AD (Corder et al., 1993; Strittmatter et al., 1993; Burns and Iliffe, 2009), whereas the pre-symptomatic phase of AD is often observed negative APOE ε4-CRP relations could also exist in young people well before the accepted AD pathological biomarkers are detectable. If so, that may indicate that any effects of lower inflammatory response in APOE ε4 carriers could accumulate throughout the lifespan.

2. Methods

Fig. 1 shows our analysis steps, datasets and sample age distributions, and analytical models used in the present study. In brief, we used our local LCBC sample for discovery and three independent external datasets (UKBB, UB-BBHI and ABCD) for replications.

2.1. Participants

The study was approved by the Regional Committee for Ethic in Medical Research in Norway. All participants provided written informed consent.

LCBC sample. Subsets of the Lifespan Changes in Brain and Cognition (LCBC) cohort (Walhovd et al., 2019) were studied. They were cognitively healthy, and well-screened for psychiatric, neurological, and health conditions known to affect the brain, but participants with common health conditions, such as moderately elevated blood pressure and those receiving hypertensive treatment were not excluded. Participants were selected based on availability of measures of cytokines, HippiV, brain Aβ or DNA genotype information. Besides high sensitivity C-reactive protein levels (CRP), nine additional cytokines were also investigated as supplementary analyses: interleukin-6 (IL6), interleukin-1 beta (IL1B), interleukin-8 (IL8), interleukin-1 alpha antagonist (IL1RA), interleukin-10 (IL10), interleukin-17A (IL17A), interferon-gamma (IFNG), tumor necrosis factor-alpha (TNFa), and monocyte chemoattractant protein-1 (MCP1, also known as CCL2). The number of unique participants varies with variables available (Fig. 1A and C, Fig. S10 and S11), ranging from N = 507 (both CRP and HippiV available; age range: 20.1–81.9 years; 329 women) to N = 749 (both APOE and > = 1 HippiV available; age range: 20–81.9 years; women: 516; men: 233). The number of unique participants and total scan observations (i.e., including longitudinal scans) for each analysis is clearly shown in Fig. 1A. Body mass index (BMI) was also collected for all LCBC participants.

COGNORM sample. Participants with CSF biomarkers, cytokine levels, and DNA genotypes from the COGNORM-study (Itilandi et al., 2017) 2012 to 2013, Oslo University Hospital and Diakonhjemmet Hospital, Oslo) were included. The COGNORM-study included 172 participants of at least 65 years of age undergoing elective gynecological, urological and orthopedic surgeries in spinal anesthesia in the two hospitals. Patients with dementia, previous stroke with sequelae, Parkinson’s disease and other conditions that probably affect cognition, were excluded. In the present study, 99 participants were included (64–93.0 years of age: 49 women; see Figs. S8 and S9 for CSF and blood marker distributions). Body mass index (BMI) was also collected for these participants.
**UK biobank population (UKBB).** Participants in the UK Biobank (UKBB) (Sugden et al., 2019) with HippV, CRP levels and DNA genotype information, were included. Ethical approval was obtained from the National Health Service National Research Ethics Service (Ref. 11/NW/0382) and all participants provided written informed consent. The dataset released February 2020 was used, including 502,507 participants, of whom 40,682 had undergone MRI scanning, 487,409 have DNA genotypes, and 468,569 had CRP measured. Participants with diagnosis of any neurodegenerative disease (N = 2,030; ICD10, G30-32), any injury to the head (N = 12,722; ICD10 S00-09), were excluded from our study. Participants with ambiguous APOE genotypes (N = 12,252), and that were genetically related with at least one other participant, were also excluded. In total, 304,322 participants (37–72 years of age; 164,303 women; 12,123 having CRP > 10 mg/L) were included to study the association of APOE ε4 with CRP levels; and 26,573 participants (45–82 years of age; 13,994 women; 1,581 having 2 MRI scans) were used to study the association of CRP and APOE ε4 with HippV.

**ABCD population.** The ABCD study aims to discover factors affecting human brain development from childhood to adolescence (Casey et al., 2018), and has included >10,000 children 9–10 years old. In the present study, we analyzed the full release 3.0, which also included brain scans for the second timepoint for a subset. There was no blood CRP measure available. In total, 10,283 unique participants (4,865 girls; 9–11 years of age), among which 3,519 have two MRI scans, were included here.

**UB-BBHI population.** Samples were obtained from participants in the Barcelona Brain Health Initiative (BBHI) (Cattaneo et al., 2018). BBHI is a population based prospective study including cognitively preserved individuals without a diagnosis of a medical neuropsychiatric condition. Inclusion in our study was based on the availability of blood CRP measures, genotypes, and MRI scans. In total, 339 individuals were included (179 women; 41–67 years of age).

### 2.2. Genotyping and imputation

**LCBC, COGNORM and UB-BBHI Sample.** Genotyping and imputation for participants of these three samples were performed previously using identical protocols (Walhovd et al., 2020). Briefly, buccal swabs were collected and genotyped using the Global Screening Array (GSA; Illumina, Inc.) with shared custom content. Pre-imputation quality check were performed using the GenomeStudio and PLINK (Chang et al., 2015) software. Samples with low call-rate (<0.95), with genotypically determined non-European ancestry, genetically related to other participants, or with abnormal heterozygosity rates, were excluded. Single nucleotide polymorphisms (SNP) with low frequencies (minor allele frequency (MAF) < 0.01), or with violation of the Hardy-Weinberg equilibrium (HWE, p < 5x10^{-6}), were excluded. Sample imputation to the HRC data (McCarthy et al., 2016) were performed using Minimac3 (Das et al., 2016) with pre-phased haplotypes estimated by SHAPEIT2 (Delaneau et al., 2011). After imputation, SNPs with MAF < 0.01, imputation R^2 < 0.6, or HWE p < 10^{-6}, were excluded from subsequent analysis.

**UK biobank population (UKBB).** Imputed genotypes for the 487,409 participants were obtained from UKBB (Category 100314). The UKBB Axiom Array from Affymetrix (about 90% of participants) and the UK BiLEVE Axiom Array (5% participants) were used for genotyping. Samples with autosome missing call-rate > 0.02, or mismatched
were obtained (https://abcdstudy.org/). Briefly, the Affymetrix NIDA data (further details see (Sugden et al., 2019)). From the HRC (McCarthy et al., 2016) and UK10K (Walter et al., 2015) imputation was performed by the UKBB center based on haplotypes genetically inferred and self-reported sex, were removed. Genotype SNPs. After quality control of these genotyped SNPs, the TOPMED study, we filtered out rare variants (MAF < 0.05), and variants showing strong deviation from Hardy-Weinberg equilibrium (p < 10^-6).

2.3. APOE genotype determination

APOE genotypes in the four datasets were determined by the two SNPs, rs429358 and rs7412. Four haplotypes (https://www.snpedia.com/index.php/APOE), TCCT, CTCT, TCTC and CTTC, of the two SNPs could not be resolved to a unique APOE haplotype, and such cases were excluded from subsequent analysis. Our present study focused on the effect of the APOE ε4 allele on CRP levels and HippV. APOE ε4 was coded as binary, i.e. carrier vs. non-carrier (low proportions of ε4 homozygotes were observed in all four samples; see Table S1 for ε4 frequencies).

2.4. Population structure

To correct for subtle population structure effects in statistical analyses, the first 10 principal components (PC) were computed using PLINK (Chang et al., 2015) for the LCBC, UB-BBHI and ABCD sample. Imputed genotypes were first quality checked by removing SNPs that have MAF < 0.05, HWE p < 10^-6, missing-rate > 0.05. Then, correlated SNPs were removed by the PLINK command --indep-pairwise 100 50 0.1. The top 10 PCs were obtained by the --pca command from PLINK. This procedure was independently performed for the LCBC, UB-BBHI and ABCD population. PCs for the UKBB population were computed by the UK biobank team.

2.5. Polygenic scores for CRP levels (PGS-CRP)

Effect sizes of genome-wide significant SNPs for CRP levels reported by Dehghan et al (Dehghan et al., 2011) and Ligthart et al (MacBean et al., 2020) were extracted from their published papers. Among 60 reported SNPs, 56 also existed in our sample. The SNP rs4420638 close to the APOE gene showed the largest effect on CRP levels and thus, was excluded in PGS computation. PGS for all participants were computed using the weighted sums methods implemented by the --score function from PLINK.

2.6. CRP determination

LCBC, COGNORM and UB-BBHI sample Circulating levels of CRP were measured by the AM-438 Quantification of CRP in Dried Blood Spots (DBS) using MSD Mesoscale ECL platform. One 3.1 mm punch from dried human whole blood samples (DBS) was eluted in 60 µl kit diluent and left standing at 4 °C overnight. After bringing the eluate to room temperature, 10 µl of the eluate were diluted in 500 µl kit diluent and analyses were performed on a MESO® QuickPlex SQ 120 Multiplex Imager using the V-PLEX Human CRP kit (K151STD-2; MSD, Rockville, Maryland, USA) as described in the manual. The lower limit of detection (LLD) was 0.08 mg/L. Samples with levels below this value were set to half of this value, 0.04 mg/L. Among the 533 samples used to discover the APOE-CRP relation, 42 had a CRP level below LLD. The number of samples with a below LLD CRP levels analyzed for the CRP-HippV relation is 83.

UK biobank population (UKBB) The serum CRP level for all participants were measured by the UK Biobank biomarker panel (http://www.ukbiobank.ac.uk/uk-biobank-biomarker-panel/). Levels of CRP were determined by the Beckman Coulter AU5800 platform (Beckman Coulter (UK), Ltd) using immune-turbidimetry. The manufacture’s detection limits were 0.08–80 mg/L. No sample had a level < 0.08 mg/L.

2.7. Hippocampal volume

LCBC, COGNORM and UB-BBHI population Participants from the LCBC and COGNORM sample were scanned at a total of four Siemens scanners at two sites; 1) Oslo University Hospital; 2) Curato, currently Aleris, Oslo: A 1.5 T Avanto equipped with a 12 channels head coil (site 1 and 2), a 3 T Skyra equipped with a 24 channels Siemens head coil (Site 1) or a 3 T Prisma equipped with a 32 channels head coil (site 1) (all Siemens Medical Systems, Erlangen, Germany). The pulse sequence used for morphometric analyses were one to two 3D sagittal T1-weighted MPRAGE sequences (Supplementary Information). All scans were reviewed for quality and automatically corrected for spatial distortion due to gradient nonlinearity (Jovicich et al., 2006) and B1 field inhomogeneity (Sled et al., 1998). Images were first automatically processed cross-sectionally for each time point with the FreeSurfer software package (version 6.0; http://surfer.nmr.mgh.harvard.edu/). The segmentation procedure automatically labels each voxel as one of 40 structures (Fischl et al., 2002), using a probabilistic brain atlas specific for the current image acquisition protocol (Han et al., 2006). Detailed description for image processing is presented in Supplementary Information. Brain scans for the UB-BBHI participants were acquired using a 3 Tesla Siemens PRISMA scanner with a 32-channel head coil and detailed protocols have been reported previously (Cattaneo et al., 2018).

UK Biobank population (UKBB). MRIs were collected and processed by the UK Biobank (https://www.ukbiobank.ac.uk) (Alfaro-Almagro et al., 2018). Imaging data were collected using 3.0 T Siemens Skyra scanners (32 channels head coil). Anatomical T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) images were obtained in the sagittal plane at 1 mm isotropic resolution, and T2 weighted FLAIR images were acquired at 1.05x1x1 mm resolution in the sagittal plane. Images were processed by the UK Biobank using the FreeSurfer 6.0 software package.

2.8. PET-Aβ status

A total of 126 participants (44.4–80.8 years of age) from the LCBC sample underwent a 18F-flutemetamol-PET scan, sensitive to Aβ accumulation (Wolk et al., 2011). Images were acquired on a General Electric Discovery PET/CT 690 scanner at Aleris Hospital and Radiology, Oslo, Norway. The PET-amyloid beta status, i.e. positive or negative, was estimated by applying principal component analysis on the standardized uptake value ratios (SUVR) from a set of 68 FreeSurfer-derived cortical regions (Mormino et al., 2014). The cut-off between groups was determined using Gaussian mixture modeling (R package mclust v5.2) for the first two principal components. We fitted 18 models, ranging from 1 to 9 mixtures, allowing for either equal or unequal variance, and selected the model with the lowest Bayesian information criterion value. As previously reported in healthy older participants (Mormino et al., 2014), the optimal model consisted of a two-distribution model with unequal variance. Participants with a > 0.5 probability of belonging to the high Aβ distribution were classified as Aβ positive, and the remaining as Aβ negative. Detailed description for PET image processing is presented in Supplementary Information.

2.9. CSF AD biomarkers for the COGNORM sample

CSF specimens were collected in polypropylene tubes at the onset of anesthesia prior to administration of the anesthetic agent. The
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specimens were centrifuged, aliquoted and stored at −80 °C (Idland et al., 2017). CSF AD biomarkers, Aβ42 and Aβ40, were measured using the Meso Scale Discovery Aβ Triplex assay (Meso Scale Discovery, Rockville, Maryland), and the ratio of Aβ42 to Aβ40 was computed. Levels of total tau (CSF-t-tau) and phosphorylated 181 total (CSF-p-tau) levels were determined using INNOTEST enzyme-linked immunosorbent assay (ELISA; Fujirebio) at the Sahlgrenska University Hospital, Sweden. CSF levels of neurofilament light protein (NFL) were determined by using a commercial ELISA (UmanDiagnostics, Umea, Sweden). Identical protocols for measuring cytokine levels as in LCBB were applied to the COGNORM sample. No data for PET-Aβ was available for this sample.

2.10. Statistical analysis

2.10.1. Effect of APOE ε4 on CRP levels

The association of APOE ε4 with CRP levels was evaluated by generalized additive models (GAM; R package ‘mgcv’). CRP levels were first log10-transformed and taken as dependent variables in the models, and a smooth age function was determined by regression splines. APOE ε4 status (binary coded; carrier [1] vs. non-carrier [0]) was set as the main predictor, and sex, BMI (to account for possible effect of BMI on CRP levels) and the top 10 PCs (to correct for subtle population stratification) were set as covariates.

2.10.2. Effects of APOE ε4 and CRP levels on HippV

To take advantage of the longitudinal brain scans available for subsets of participants, generalized additive mixed models (GAMM; R package ‘mgcv’) were used to model the nonlinear relation between HippV and age, taking into account intra-individual correlations. APOE ε4 status, estimated intracranial volume (ICV), sex, and the top 10 PCs were coded as fixed effects, and participant unique identifiers were set as the random effect. In addition, we allowed different smooth age functions for the two APOE ε4 groups. Standardized HippV (mean zero and standard deviation one) was set as the dependent variable. To estimate the effect of CRP levels on HippV, instead of APOE ε4 status, the log10-transformed CRP levels were set as the main predictor. Moreover, both CRP levels and APOE ε4 status and the interaction term between the two were used as main predictors to test whether the two variables contribute to HippV variation independently. All statistics reported are on the log10 scale of CRP levels. Bonferroni correction was applied to account for multiple testing.

2.10.3. Effects of APOE ε4 and CRP levels on PET-Aβ status and CSF marker levels

Logistic regression analyses were performed to estimate the effect of APOE ε4 and CRP levels on PET-Aβ status. PET-Aβ status was used as dependent variable; APOE ε4 status and CRP levels were set as the main predictor, separately. Age at measurement, sex, BMI, and the top 10 PCs were used as covariates. Linear regression models were then used to estimate the effect of APOE ε4 and CRP levels on CSF marker levels. Each marker was analyzed separately. In each model, the CSF marker level was inverse-normal transformed to have zero mean and one standard deviation and was used as dependent variables. APOE ε4 and CRP levels were used as the main predictor, separately. Age at measurement, sex, BMI were used as covariates. Here, False Discovery Rate (FDR) was used for multiple testing correction (Benjamini and Hochberg, 1995).

2.11. Replication analysis

Corresponding models for the relation between APOE ε4 and HippV were applied in the UKBB and ABCD replication samples. For the UB-BBHI sample, which had no longitudinal brain scans, corresponding GAM models were used.

3. Results

The LCBB sample had a wider age-range than the other samples (Fig. 1B), but had fewer participants aged 35 to 65 years. The UKBB sample had higher CRP levels than those of the LCBB and UB-BBHI samples (Fig. S1, two-sided t test, p < 2x10⁻¹⁶). ABCD participants had smaller HippV than those in the other three samples, and the UB-BBHI participants had the largest HippV among the four (pairwise two-sided t tests, Bonferroni-corrected p < 0.05; Fig. S2). The LCBB sample has a higher frequency of APOE ε4 than the other three samples; but, the ε4 frequencies in all four samples are consistent with a recent report of geographical frequency differences (Table S1) (Kern et al., 2015).

3.1. Stronger genetic control over lower CRP levels

To investigate if the genetic contributions to CRP levels vary with blood CRP levels, we stratified CRP levels into 5 bins and regressed log10 transformed CRP levels in each bin on APOE ε4 status, including age and sex as covariates in the models. The false discovery rate procedure was used for multiple testing correction (along test with the full CRP range in each dataset, 18 tests performed). We found that whereas ε4 was associated with CRP levels overall (bin: All, FDR-corrected p < 0.05), only in the bin with lowest CRP level ranges (<1mg/L) was ε4 nominally associated with lower CRP level in the LCBB and the UB-BBHI samples (FDR-corrected p = 0.09 and 0.02, respectively; Fig. 2A and Table S2). To test whether this was due to too few participants having high CRP levels in the two healthy samples, we performed the same analysis for the UKBB dataset (N = 304,322). The results clearly showed that the higher the CRP levels, the less significant was the association between APOE ε4 and CRP levels (Fig. 2A-B).

We next regressed the log10 transformed CRP levels in each bin on the PGS-CRP (constructed without APOE-region SNPs). The same covariates as used above for APOE ε4 were also included in these models. PGS-CRP showed a weaker effect on CRP levels than for APOE ε4 (Fig. 2B, Table S3), and PGS-CRP values were only positively associated with overall CRP levels (bin: [0–100] mg/L) in both the LCBB and UB-BBHI sample (FDR-corrected p < 0.05, Table S3). Whereas, due to large samples in each bin, the UKBB showed significant association for the three bins with lower CRP levels (FDR-corrected p < 0.05, Fig. 2B). Thus, the same trend as observed for ε4 was also apparent for PGS-CRP – the higher the CRP levels, the less their variations were attributable to genetic variation.

3.2. APOE ε4 is associated with lower CRP levels across lifespan

Across the whole lifespan, we observed a strong and consistent negative association between APOE ε4 and CRP levels (Fig. 2C). Specifically, in the LCBB sample – where 54% of participants were below 40 years of age – APOE ε4 carriers were found to have lower CRP levels on average across the lifespan (N = 533; beta = -0.15, p = 7.9x10⁻⁸) as compared to non-carriers. When the analysis was restricted to those < 40 years of age, similar effect sizes were obtained but not statistically significant (N = 289; beta = -0.10, p = 0.12), possibly due to reduced sample size. In the first replication dataset (UB-BBHI, N = 339), we also found a strong negative effect of ε4 on CRP levels that existed across the whole age-range (41–67 years; beta = -0.21, p = 2.2x10⁻⁵). The concave curve in Fig. 2C for UB-BBHI may be due to too few individuals having the ε4 allele (N = 69). In the very large UKBB dataset, the effect size of ε4 became smaller but more significant (Fig. 2C, N = 304,322; APOE ε4 carrier vs non-carrier: beta = -0.13, p < 2x10⁻¹⁶). Although we always modelled the age function by regression splines, in this large UKBB sample, CRP levels were almost linearly increasing with age (approximated p for age < 2x10⁻¹⁶). The positive associations between CRP levels and age were also significant in the LCBB and UB-BBHI sample (p < 0.05) (Fig. 2C).
To corroborate the linear regression results on disjoint bins on CRP levels in Fig. 2B, we performed GAM models on inclusive bins on CRP levels (>5 mg/L, >7 mg/L and >10 mg/L) using the UKBB sample. While APOE ε4 was associated with CRP levels that is below 10 mg/L (N = 29,219; beta = -0.12; p < 2x10^{-16}) it was not associated with CRP levels in any of the three groups (>5 mg/L, N = 34,303; beta = -0.0064; p = 0.04; >7 mg/L, N = 20,880, beta = -0.002, p = 0.6; >10 mg/L, N = 12,123; beta = 0.004; p = 0.36) after multiple testing correction.

### 3.3. APOE ε4 is associated with smaller hippocampal volumes

We observed a negative association between APOE ε4 and HippV (Fig. 3). In the LCBC sample (N = 749, N-MRI scans = 1,641, 20–81 years of age), APOE ε4 carriers had a lower hippocampal volume on average across the lifespan as compared to non-carriers (beta = -0.17, p = 2.97x10^{-3}). In our first replication sample (UB-BBHI), ε4 showed the same direction of effect on HippV but was not significant (Fig. 2; p = 0.67). However, in the other two replication samples – including the childhood ABCD sample (UKBB, N = 26,573, N-MRI scans = 28,154; ABCD, N = 10,283, N-MRI scans = 13,802) – ε4 was significantly associated with smaller HippV on average across age (UKBB, beta = -0.02, p = 0.025; ABCD, beta = -0.04, p = 0.027). We also tested the APOE ε4 by age interactions in the UKBB sample using linear mixed models and detected no significant interacting effects (Supplementary Information, Fig. S12).

### 3.4. High CRP levels associate with small hippocampal volumes

CRP levels showed stronger association with smaller HippV than did APOE ε4 status (Fig. 4). In the LCBC sample (N = 507, N-MRI scans = 1,344, 20–81 years of age), increased CRP levels associated with small hippocampi (beta = -0.17, p = 1.21x10^{-3}). Including APOE ε4 status as an additional covariate did not qualitatively change this association. In UKBB, higher CRP levels were also associated with smaller HippV (beta (Supplementary Information, Fig. S12)).

![Fig. 2. Associations between APOE ε4, PGS-CRP and CRP levels. Associations between APOE ε4 (carrier vs non-carrier) (A), PGS-CRP (B) with CRP levels, divided into six bins based on measured CRP levels (mg/L): <1, 1–3 mg/L; 3–5 mg/L; >5 mg/L; >7 mg/L (C) Association between APOE ε4 and CRP levels across age range and independent samples. Fitted CRP levels were obtained by fitting generalized additive model (GAM), which model age by regression splines, and plot against age at measurement. Colors indicate ε4 status. Names of datasets and their sizes are shown on top of each panel.](image)

![Fig. 3. Associations between APOE ε4 and hippocampal volume. Fitted total hippocampal volumes by GAMM (LCBC sample (Disc_ LCBC), UK Biobank subsample (Rep2_UKBB) and the ABCD sample (Rep3_ABCD) and by GAM (Rep1 UB-BBHI) were plotted against baseline age (x axis). Hippocampal volumes were stratified by APOE ε4 status (carrier: positive; non-carrier: negative). Model fitting includes sex, age at measurement (smoothing variable in GAM (M), x axis), BMI and the top 10 principal components as covariates. For GAMM, random intercepts were model by taking subject identifiers as random term. Sample sizes were shown on the top of each panel.](image)
3.5. APOE ε4 and CRP levels in relation to PET-Abβ status and CSF biomarker levels

APOE ε4 carriers exhibited increased levels of amyloid plaque in the brain (N = 126, t = 3.33, FDR-corrected p = 1.30x10^{-5}), decreased levels of CSF-Ab42 (t = -2.90, FDR-corrected p = 7.438x10^{-5}), and decreased CSF-Ab42 to CSF-Ab40 ratio (t = -5.15, FDR-corrected p = 5.81x10^{-5}) (Fig. 5). We did not observe associations between APOE ε4 and other AD biomarkers (Table 1), though we did find positive trends between CRP levels and CSF-Ab42 and CSF-Ab42 to CSF-Ab40 ratio at borderline significance (uncorrected p = 0.06 and 0.03, respectively). However, this association was largely accounted for by the effect of the APOE ε4 status (after including APOE ε4 status as an additional covariate the association disappeared).

3.6. PGS-CRP was not associated with AD markers

Because the APOE gene has been associated with CRP levels in several previous studies, we tested if genetically predicted CRP levels (excluding APOE-region SNPs) could predict AD biomarkers in the three samples. There were no associations between PGS-CRP and any AD biomarkers studied. In addition, there were no associations between PGS-CRP and APOE ε4 status (Fig. S3). Whereas these results may suggest the effect of CRP on AD biomarkers was largely driven by APOE ε4, it should be noted that both CRP levels and APOE ε4 were significantly associated with HippV in the multivariate model.

4. Discussion

By using four complementary populations comprising an age-span of 9–90 years (total N = 315 753) we showed that APOE ε4 was continuously and consistently associated lower circulating CRP levels. This association is mainly for CRP levels in the range of normal physiological conditions (CRP < 3 mg/L). Our findings extend previous knowledge by indicating that the association exists before the typical age at which accepted AD biomarkers can be observed (~40 years) (Selkoe and Hardy, 2016) and continues beyond the typical age of onset for AD (~60 s). Moreover, we demonstrated both APOE ε4 and higher CRP levels are associated with smaller hippocampal volumes, a marker that has frequently been linked to low cognitive performance and high risk of AD. Whereas APOE ε4 was clearly associated with PET-Abβ and CSF markers for AD, CRP did not show convincing associations with any of
The effects of APOE ε4 (carriers vs. non-carriers) and CRP levels PET-amyloid beta load in the brain and CSF marker levels were evaluated by general linear models, including age at measurement, BMI, sex and the top 10 genetic principal components as covariates (APOE related analysis only). Logistic regression models were used for PET-Aβ positiveness, and linear regression models were used for other markers, which have been normalized before modeling. Sample population was used for PET-Aβ components as covariates (APOE related analysis only). Logistic regression was used for other markers, which have been normalized before modeling. Sample population was used for PET-Aβ components as covariates (APOE related analysis only). Logistic regression was used for other markers, which have been normalized before modeling. Sample population was used for PET-Aβ components as covariates (APOE related analysis only). Logistic regression was used for other markers, which have been normalized before modeling. Sample population was used for PET-Aβ components as covariates (APOE related analysis only). Logistic regression was used for other markers, which have been normalized before modeling. Sample population was used for PET-Aβ components as covariates (APOE related analysis only). Logistic regression was used for other markers, which have been normalized before modeling.

| Marker          | APOE ε4 | CRP |
|-----------------|---------|-----|
|                | N t-score | p  | N t-score | p  |
| PET-Aβ          | 126 3.33 | 0.89x10⁻⁴ | 98 −1.52 | 0.30(1.0) |
| CSF-Aβ40        | 59 0.11 | 0.91(1.0) | 66 0.49 | 0.63(1.0) |
| CSF-Aβ42        | 59 −2.90 | 5.31x10⁻⁵ | 67 1.91 | 6.04x10⁻² |
| CSF-Aβ42/A40    | 59 −5.15 | 3.63x10⁻⁴ | 66 2.17 | 3.37x10⁻¹ |
| CSF-t-tau       | 59 1.45 | 0.15(1.0) | 67 −0.45 | 0.66(1.0) |
| CSF-p-tau       | 59 1.05 | 0.30(1.0) | 67 −0.39 | 0.70(1.0) |
| CSF-NFL         | 59 1.26 | 0.21(1.0) | 67 −0.51 | 0.61(1.0) |

Table 1: Effects of APOE ε4 and whole blood CRP levels on brain Ajβ load and CSF biomarkers.
We replicated our findings in three independent datasets with varying age ranges, sizes, and data acquisition platforms, indicating the results are robust. We also accounted for the effect of BMI by including it as a covariate in the models. A limitation is that, due to the high cost of measuring AD biomarkers, only a small number of participants had PET-AP and CSF markers in our sample. That we did not find associations between APOE ε4 and CSF-Aj40, CSF-NFL and CSF tau levels might therefore be due to low statistical power, and it remains to be seen whether future larger scale studies may identify such associations. In addition, for a subset of participants (COGNORM study), cytokine levels were measured three years later than the brain scan. However, such a complication does not affect our conclusions.

5. Conclusion and perspectives

By synthesizing four cognitively healthy populations with wide age spans, we observed that APOE ε4 was associated with low whole blood/serum CRP levels across the lifespan. While a relationship between acute inflammation and dementia risk has been established (Komaroff, 2020), we think in a lifespan perspective, further investigations of the role of very low-grade inflammatory responses may also be worthwhile. Based on our findings, it may be fruitful in further research to focus on whether APOE ε4 can confer risk by being associated with a lower inflammatory response to daily exposures, possibly to greater accumulation of low-grade inflammatory stress though the lifespan. Our study shows previous interpretations of these associations are incomplete, and future mechanistic studies are needed to resolve this intriguing relation.

Author contributions

YW, AMF and KBW conceived and designed the study. JMR, MP, FM, AP, JMT, MC, DB, JMY, IKA, AMF, KBW, LOW and AVI collected sample and performed MRI and cytokine measurements. YW performed statistical analysis. All authors contributed to the interpretation of the results. YW and KBW wrote the draft. All authors edited and approved the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2021.12.008.

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