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DNA methylation age acceleration mediates the relationship between systemic inflammation and cognitive impairment

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Abstract

Background: Chronic inflammation and DNA methylation are potential mechanisms in dementia etiology. The linkage between inflammation and DNA methylation age acceleration in shaping dementia risk remains understudied. We explored the association of inflammatory cytokines with cognitive impairment and whether DNA methylation age acceleration mediates this relationship.

Research design and methods: Using data from the 2016 Health and Retirement Study (n = 3,346, age >50), we estimate the associations between each inflammatory cytokine (interleukin-6

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Ethical declaration

These analyses of existing data were approved by the University of Michigan Institutional Review Board (HUM00128220).

(IL-6), C-reactive protein (CRP), and insulin-like growth factor-1 (IGF-1)), and cognitive status, classified using the Langa–Weir method. We tested if DNA methylation age acceleration mediated the relationship between systemic inflammation and cognitive impairment, adjusting for sociodemographic, behavioral factors, chronic conditions, and cell-type proportions.

Results: Cognitive impairment prevalence was 16%. A doubling of IL-6 was associated with a 12% higher odds of cognitive impairment (OR = 1.12, 95% CI: 1.02–1.22), and 0.77 years of GrimAge acceleration (95% CI: 0.64–0.90). Similar associations were found for CRP and IGF-1. Mediation analysis indicated that 17.7% (95% CI: 7.0–50.9%) of the IL-6–cognitive impairment association was mediated by the GrimAge acceleration. Comparable mediated estimates were found for CRP and IGF-1.

Conclusions: Systemic inflammation is associated with cognitive impairment, with suggestive evidence that this relationship is partially mediated through DNA methylation age acceleration.

Keywords

Cytokines; GrimAge; epigenetic clocks; cognition; DNA methylation; dementia; inflammation

1. Introduction

The risk of cognitive impairment in aging adults is influenced by various health determinants [1–3]. Peripheral immunity may be related to cognitive impairment in older adults [4,5]. Molecular mechanisms, such as DNA methylation age acceleration, may contribute to poor cognitive health outcomes, including cognitive impairment [6]. Therefore, unveiling the potential shared contributions of the immune response and the cellular mechanisms associated with accelerated epigenetic aging could inform public health efforts to identify individuals at greater risk for cognitive decline.

The immune profiles of aging adults are characterized by a state of immune dysregulation with high circulating blood levels of pro-inflammatory cytokines in the absence of tissue triggers – a process often referred to as *inflammaging* [7]. Elevated levels of chronic inflammation are associated with multiple age-related diseases, such as coronary heart disease, stroke, diabetes, kidney disease, and dementia [2,8–11]. High levels of pro-inflammatory cytokines such as C-reactive protein (CRP), Interleukin-6 (IL-6), Interleukin-1 (IL-1), and Tumoral Necrosis Factor alpha (TNF-α) have been found in atherosclerotic plaques of coronary arteries, tubular epithelial cells of the kidney tissue, and in brain parenchyma post-ischemic stroke [7]. The co-localization of pro-inflammatory cytokines in tissue-specific processes suggests that systemic inflammation is an underlying mechanism of multiple chronic diseases and a biological signature of accelerating aging. However, whether inflammaging contributes to aging-related pathologies, such as those leading to cognitive impairment, remains uncertain.

To better understand the role of systemic inflammation in the etiology of age-related diseases, we need to assess the link between the inflammatory response and the molecular mechanisms implicated in accelerated aging [7,12,13]. DNA methylation clocks are biomarkers of biological aging [14]. These clocks are built from machine learning

algorithms that identify patterns of specific DNA methylation sites in different tissues, which are then used to predict the DNA methylation age of the sample source (15,16). The residual from a regression of an individual's estimated DNA methylation age and their chronological age is a measure of accelerated biological aging. The first generation of DNA methylation clocks was designed to estimate chronologic age and the second generation of DNA methylation clocks sought to characterize phenotypic age, which is of primary interest in this study. Premature biological aging, as measured by the second-generation DNA methylation clocks, has been associated with several chronic conditions, early mortality, and cognitive decline [15,16]. To date, epidemiological studies exploring the interplay between blood circulating levels of cytokines, DNA methylation clocks, and aging-related diseases are limited.

In a cross-sectional sample of the Health and Retirement Study, a population-based cohort of older adults in the United States, we tested the hypothesis that elevated circulating blood levels of cytokines and accelerated DNA methylation aging would be associated with prevalent cognitive impairment. We used mediation analysis to estimate to what extent the effect of each cytokine on cognitive impairment was mediated through DNA methylation age acceleration.

2. Methods

2.1. Study population

Data were obtained from the US Health and Retirement Study (HRS), a population-based longitudinal study of aging adults sponsored by the National Institute on Aging (NIA U01AG009740). To ensure a representative sample of the demographic composition of the US, the HRS oversamples non-Hispanic Black and Hispanic participants employing a multistage probability design [17]. The original HRS cohort began in 1992, and participants were interviewed every 2 years. The study interview collects data on sociodemographic, economic, behavioral, psychological, and other health-related factors thought to be associated with the aging process [18]. In 2016, all participants who completed the interview were asked to consent to participate in a Venous Blood Study, except for proxy participants and those living in a nursing home. A panel of seven cytokines and other markers of cellular aging were assessed in subsamples of the Venous Blood Study [19]. The participants provided written informed consent at the time of the data collection. These secondary data analyses were approved by the University of Michigan Institutional Review Board (HUM00128220).

In a subsample of the 2016 Venous Blood Study, we tested the cross-sectional associations between seven cytokines (exposures) and six DNA methylation clocks (mediators); and their respective associations with prevalent cognitive impairment (outcome). In total, 9,934 participants from the HRS study were selected for the venous blood study in 2016, and 3,556 participants had complete information on cytokines, DNA methylation clocks and APOE-*ε4* allele status. Among these, 3,450 had complete demographic information. Additional 101 participants were excluded due to self-reporting their race/ethnicity as "non-Hispanic Other." The final sample included 3,346 participants, and participant inclusion is visualized in a flow chart (Supplemental Figure S1). This study adhered to both

the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [20] and the Guideline for Reporting Mediation Analyses (AGReMA) [21].

2.2. Cytokine assays

Cytokine assays were conducted on the Venous Blood Study sample of the 2016 HRS wave. Blood collection was managed by Hooper Holmes Health & Wellness. Approximately 50.5 mL of blood was collected in 6 tubes – one 8 mL CPT tube, three 10 mL double gel serum separator tubes (SST), one 10 mL EDTA whole-blood tube, and a 2.5 mL PAXgene RNA tube. The SST tubes were centrifuged in the field before being shipped overnight to the Advance Research Diagnostic Laboratory at the University of Minnesota. Tube processing was done within 24 hours of arrival at the lab or within 48 hours of collection. A panel of seven cytokines (CRP, IL-6, IL-1RA, IL-10, sTNF-R1, IGF-1, and TGF-β1) were measured in the serum via enzyme-linked immunosorbent assay (ELISA). Detailed information on each cytokine assay procedure can be found elsewhere [19].

For the statistical analyses, we used cytokines as continuous variables. Given their positive skewness and the wide range of cytokine levels across participants, we used the log2 transformation of CRP, IL-6, IL-1RA, IL-10, sTNF-R, and the square root of IGF-1 to normalize their distributions. We considered patterns among proinflammatory cytokines (CRP, IL-6, IL-10, and sTNF-R1) and among anti-inflammatory cytokines (IGF-1 and TGF-β1).

2.3. DNA methylation assays and DNA methylation age acceleration measures

DNA methylation was collected and processed by the HRS [19]. DNA methylation was measured in a nonrandom subsample (n = 4,104) of participants who consented to participate in the Venous Blood Study, and a total of 4,018 (97.9%) samples passed the quality control assessment. This subsample was representative of the sociodemographic distribution of the HRS. Whole-genome DNA methylation data were assessed using the 850K MethylationEPIC BeadChip (Infinium) microarray (Illumina Inc.) [19] at the University of Minnesota. The samples were randomized across plates by key demographic variables such as age, cohort, sex, education, and race/ethnicity. The R package *minfi* [19,22] was used to preprocess the RGChannelSet object and perform quality control. Beta methylation values were calculated. Sample mismatches in sex and any control (cell lines, blind duplicates) were removed (n = 58). For any given participant, probes with detection p-values <0.01 were imputed by the HRS team to the mean methylation value of the given probe across all samples before public release [19]. In addition, the HRS team applied the ComBat method to account for technical variances due to factors such as batch and plate effects [19].

The HRS calculated and released data for several epigenetic clocks. A DNA methylation clock is an estimator built from DNA methylation CpG sites that are strongly correlated with age [15]. DNA methylation clocks are generally categorized as: a) chronological, or clocks that provide a “forensic” estimation of chronological age (i.e., Horvath, Hannum, HorvathSkin); and b) phenotypical, or clocks that are informative of a disease state (i.e., GrimAge, Levine, and the methylation pace of aging clock or DunedinPoAm) [15]. For the

purpose of our analysis, we used these six clocks and explored their relationship with both inflammatory cytokines and cognitive impairment. In our mediation analysis, we prioritized the phenotypic clock GrimAge for its strong *a priori* relationship with both cytokines and cognitive impairment [23]. The GrimAge clock consists of approximately 1,030 CpGs and was built using smoking pack-years and seven plasma proteins associated with mortality risk [24]. Horvath is a multi-tissue clock consisting of 353 CpGs and an average error of 3–5 years [25]. The Hannum clock reflects age-related changes in cell compositions and consist of 71 CpGs [26]. Despite sharing only 6 CpG sites, Horvath and Hannum clocks are highly predictive of all-cause mortality [16]. The skin and blood clock (HorvathSkin) was built from 391 CpGs, and shares 71 CpGs with the Hannum and 60 CpGs with the Horvath clock, this clock was developed to measure the age of human fibroblasts, keratinocytes, buccal cells, endothelial cells, skin and blood samples [27]. The Levine clock, also known as PhenoAge, was built from 513 CpGs that predict a composite score of biological aging based on diverse clinical parameters [28]. Finally, the age-38 DunedinPoAm estimator was built to predict longitudinal changes in 18 physiological markers of organ function [29]. We transformed the DunedinPoAm rate into years for alignment with the other age estimators.

DNA methylation age acceleration represents the residuals of the relationship between the estimated DNA methylation age and the observed chronological age [6]. This variation reflects an individual's rate of aging [16]. To estimate age accelerated residuals, we regressed each DNA methylation clock (Y) on participants' chronological age (X) at the HRS 2016 wave. DNA methylation age accelerated residuals were inspected to meet linear regression assumptions (e.g., normality and constant variance of the errors). Predicted residual values greater than zero reflect DNA methylation age acceleration and negative or zero residual values reflect non-accelerated DNA methylation age. In statistical analysis, we used epigenetic age accelerated residuals in their continuous form.

2.4. Cognitive status

We used cognitive data from 2016 to maximize sample size and analytic power. All participants in our study were non-proxy respondents. Cognitive function was assessed through a series of telephone interview cognition questions [30], which provides a total score of 0 to 27 points based on a battery of cognitive tests that include a 10-word immediate and delayed recall test, a serial 7 subtraction, and counting backwards. This measure has been validated and shown to have high sensitivity and specificity for identifying cognitive impairment and dementia [31–33]. Participants' cognitive status was categorized using the Langa-Weir algorithm, which was developed based on the Aging, Demographics, and Memory Study and has demonstrated strong agreement with clinical diagnoses [34,35]. Cognitive status was classified as dementia (0 to 6 points), cognitive impairment non-dementia (7 to 11 points), and normal cognition (12 to 27 points) [36]. In statistical analyses, we compared dementia and cognitive impairment non-dementia versus normal cognition separately. To leverage a larger number of cases and higher statistical power to detect potentially mediated effects in mediation analysis, we combined dementia and cognitive impairment non-dementia into a single category representing cognitive impairment and compared it to normal cognition.

2.5. Covariate measures

Our analysis adjusted for potential confounders in the association between inflammatory biomarkers and cognitive impairment, and between DNA methylation age acceleration and cognitive impairment. Sociodemographic and genetic confounders included: age at the interview date (continuous), self-reported sex (female, male), self-reported racialized social group (Hispanic, non-Hispanic Black, non-Hispanic White), self-reported highest educational attainment (more than college, college or some college, high school or less), and *APOE-ε4* allele carrier status (at least 1 copy, no copy) [37]. Genetic information on *APOE-ε4* allele status was obtained from phased genetic data imputed to the worldwide 1000 Genomes Project reference panel. Genotyping and imputation information for the HRS is available elsewhere [38]. Health behavioral confounders included: self-reported body mass index (BMI; calculated as weight in kilograms divided by height in meters squared, continuous), weekly vigorous, moderate, or light physical activity (yes, no), alcohol consumption (reported as the number of drinks a day when drinks, with nondrinkers assigned a value of 0, continuous), and smoking status (current, former, never) [39]. Chronic health conditions included self-reports of a physician diagnosis of each of the following conditions: high blood pressure (yes, no), diabetes (yes, no), cancer (yes, no), lung disease (yes, no), heart disease (yes, no), stroke (yes, no), psychiatric problems (yes, no), and arthritis (yes, no); operationalized in our models as a categorical variable (0, 1–2, 3 or more conditions) [40]. For the relationship between inflammatory biomarkers and DNA methylation age acceleration, we adjusted for age, sex, racialized social group, and highest educational attainment as mentioned above. Additionally, we adjusted for cell-type proportions (percent granulocytes and percent lymphocytes measured from complete blood count measures, both continuous variables), as differences in cell-type distribution can influence DNAm age independently of intrinsic biological aging. This adjustment helps capture cell-intrinsic aging rather than variation due to immune cell shifts or inflammation [40]. In the mediation analysis, we adjusted for all these covariates in our model.

2.6. Statistical analysis

We compared the covariate distributions between the included and excluded HRS participants due to missing data. First, in bivariate analysis, we examined the distribution of covariates by cognitive status (dementia, cognitive impairment non-dementia, and normal cognition) using the one-way ANOVA test for continuous variables and the chi-square test for categorical variables. We estimated the Pearson correlation between cytokines, epigenetic clocks, and cell-type proportions and visualized them in correlation plots.

2.6.1. Cytokine and cognitive status regression models: We employed multivariable-adjusted logistic regression analysis to estimate the association between each cytokine exposure and the odds of each of prevalent dementia and cognitive impairment non-dementia relative to normal cognition. A fully adjusted logistic regression model was built for each combination of cytokine and cognitive status. This model included adjustments for sociodemographic factors (age, sex, racialized social group, education), *APOE-ε4* carrier status, health behaviors (body mass index, smoking status, alcohol consumption, exercise), and chronic conditions [40]. We reported the odds ratio (OR) and 95% confidence interval (CI) for each cytokine. To account for multiple comparisons, we

calculated false discovery rates (FDR) [41], considering an FDR value of less than 0.1 as significant.

2.6.2. Cytokines and DNA methylation age acceleration models: We employed multivariable linear regression analysis to estimate the associations between each cytokine and each DNA methylation age acceleration measure. We built three generalized linear models to estimate the relationship between each cytokine with each phenotypic clock (GrimAge, Levine, DunedinPoAm) and each chronologic clock (Horvath, Hannum, HorvathSkin). We reported the effect estimates and 95% CI for each cytokine and calculated the FDR.

2.6.3. DNA methylation age acceleration and cognitive status models: We used multivariable logistic regression analyses to estimate the associations between each DNA methylation clock and cognitive status. In this analysis, we adjusted for sociodemographic factors (age, sex, racialized social group, education), *APOE-ε4* carrier status, cell-type proportions, health behaviors (body mass index, smoking status, alcohol consumption, exercise), and chronic conditions. We reported the OR and 95% CI for cognitive impairment associated with a 1-year increase of DNA methylation age acceleration and calculated the FDR.

2.6.4. Mediation analysis: We employed regression-based causal mediation analysis [42,43] to investigate whether DNA methylation age acceleration mediated the total effect of cytokine levels on cognitive impairment (dementia and cognitive impairment non-dementia) relative to normal cognition [44]. For our mediation models, we selected three different cytokines (CRP, IL-6, and IGF-1) as exposures, because these biomarkers were strongly associated in the multivariable models with both dementia and cognitive impairment non-dementia. We also selected GrimAge acceleration as a mediator because this clock was the most strongly associated in multivariable logistic regression models with impaired cognitive status. To leverage a larger sample size and statistical power, we combined dementia and cognitive impairment non-dementia cases into a single category representing cognitive impairment, our outcome.

We built three different mediation models with C-reactive protein, interleukin-6, and IGF-1 as the primary predictor separately. We used the *CMAverse* R studio package to estimate the proportion of the total effect of each cytokine on cognitive impairment that was mediated through the GrimAge clock [42,43]. The *CMAverse* accommodates for different generalized linear models within the mediation analysis. For example, logistic regression was employed to estimate the exposure–outcome relationship. The exposure–mediator relationship was estimated through linear regression analysis, and the mediator–outcome relationship was estimated through logistic regression.

In addition, because our primary interest in this mediation analysis was to estimate the mediated effect of epigenetic age on the relationship between cytokine exposure and cognitive impairment, we calculated mediational e-values to understand to what extent an unmeasured confounder could nullified the observed mediational proportions [45]. All

statistical analyses were conducted using R statistical software (version 3.6.2). Code to perform the analyses is available (https://github.com/bakulskilab).

2.6.5. Sensitivity analysis: To explore potential sex differences, we conducted sensitivity analyses to examine all models stratified by sex. This included (1) linear regression models of cytokines on DNA methylation age acceleration, (2) logistic regression models of cytokines and DNA methylation age acceleration on cognitive impairment, and (3) regression-based mediation analyses with DNA methylation age acceleration as the mediator of the cytokine and cognition association.

3. Results

3.1. Sample characteristics

Our study included a cross-sectional sample of 3,346 participants of the 2016 venous blood study in the HRS cohort (Supplemental Figure S1). Most exclusions were due to missing biomarker data, particularly DNA methylation measures. The included sample participants were older and had a relatively higher prevalence of chronic conditions compared to the excluded sample, while other characteristics were generally similar across both groups (Supplemental Figure S1). On average, the included participants were 70 (SD = 9.6) years of age, 58% female, 70% had completed high-school education or less, and 17% of participants were racialized as non-Hispanic Black and another 14% as Hispanic (Table 1). The prevalence of dementia was 3.7% (n = 125) and the prevalence of cognitive impairment non-dementia was 16% (n = 543). Elevated levels of pro-inflammatory cytokines such as CRP, IL-6, IL-10, and sTNF-R1 were observed among cognitively impaired participants, but the differences were not statistically significant (Table 1 & Figure 1). In contrast, higher levels of anti-inflammatory cytokines such as IGF-1 and TGF-β were observed in cognitively normal participants. There were no differences in the distributions of IL-1ra across the different cognitive categories (Table 1). Higher GrimAge acceleration was observed among participants with dementia (1.0 years) and cognitive impairment non-dementia (1.13 years), relative to those with normal cognition (−0.27 years) (Table 1).

Proinflammatory cytokines (i.e., CRP, IL-6, IL-10, and sTNF-R1) were weakly to moderately correlated (Supplemental Figure S2). Among all the cytokines, CRP and IL-6 exhibited the strongest correlation (r = 0.44). The anti-inflammatory cytokine IGF-1 was negatively correlated with pro-inflammatory cytokines (i.e., IL-10, IL1-ra, sTNF-R1, CRP, IL-6) (Supplemental Figure S2). The phenotypical clocks GrimAge and DunedinPoAm had the strongest correlation coefficient (r = 0.62) and the chronological clocks such as Horvath, Hannum, and HorvathSkin were moderately correlated (Supplemental Figure S2).

3.2. Adjusted association between cytokines and cognitive status

In general, proinflammatory cytokines were associated with higher odds of prevalent dementia and cognitive impairment non-dementia. For example, a doubling of CRP was associated with a 1.16 (95% CI: 1.01, 1.32) times greater odds of prevalent dementia with a statistically significant FDR adjusted p-value, and 1.07 (95% CI: 1.00, 1.15) times higher odds of cognitive impairment non-dementia (Table 2). Similarly, a doubling of IL-6 was

associated with 1.19 (95% CI: 1.00, 1.41) times greater odds of prevalent dementia and 1.12 (95% CI: 1.02, 1.22) times greater odds of cognitive impairment non-dementia with significant FDR adjusted *p*-value. In contrast, the anti-inflammatory cytokine IGF-1 was associated with lower odds of prevalent dementia (OR = 0.88; 95% CI: 0.78, 0.98) with a statistically significant FDR adjusted *P*-value and cognitive impairment non-dementia (OR = 0.94; 95% CI: 0.89, 1.00). Other cytokines were not associated with cognitive status (Table 2).

3.3. Association between cytokines and DNA methylation age acceleration

In multivariable adjusted models, cytokines were highly associated with DNA methylation age acceleration, and pro-inflammatory cytokines were correlated with increased age accelerated residuals (Supplemental Tables S2 & S3). Exposure to anti-inflammatory cytokines was associated with decreased DNA methylation age acceleration. In our sample, a doubling of the CRP was associated with 0.69 (95% CI: 0.54, 0.85) years of Levine age acceleration (ß = 0.10 SD unit of Levine age acceleration, 95% CI: 0.08, 0.12), 0.56 (95% CI: 0.47, 0.66) years of GrimAge age acceleration (ß = 0.12 SD unit of GrimAge age acceleration, 95% CI: 0.10, 0.14), and 0.77 (95% CI: 0.64, 0.90) years of DunedinPoAm age acceleration (ß = 0.12 SD unit of DunedinPoAm age acceleration, 95% CI: 0.10, 0.14), all with significant FDR adjusted *p* values. Similar patterns of association were observed between CRP and chronologic age clocks (Horvath, Hannum, and HorvathSkin) (Supplemental Table S3 & Supplemental Tables S2–S3). Interleukin-6, IL-1RA, IL-10, and sTNF-R1 were also strongly associated with higher DNA methylation age acceleration across chronological and phenotypical clocks (Supplemental Table S3 & Supplemental Tables S2–S3). In contrast, higher levels of the anti-inflammatory IGF-1 were associated with lower age acceleration across all phenotypic, but not the chronologic clocks. For example, a one unit increase in the square root-transformed IGF-1 levels was associated with a −0.19 (95% CI: −0.32, −0.07) lower years of Levine age acceleration, −0.14 (95% CI: −0.22, −0.06) lower years of GrimAge acceleration, and −0.32 (95% CI: −0.42, −0.21) lower years of DunedinPoAm acceleration (Supplemental Table S3 & Supplemental Tables S2–S3). TGF-β was associated with the DudeninPoAm clock. A doubling of TGF-β was associated with 0.02 (95% CI: 0, 0.03) years of the DunedinPoAm age acceleration.

3.4. Association between DNA methylation age acceleration and cognitive status

In multivariable logistic regression models, phenotypic clocks were associated with dementia and cognitive impairment non-dementia, but not chronological clocks. A one-year increase in GrimAge acceleration was associated, though not FDR significant, with 1.01 (95% CI: 0.96, 1.07; OR = 1.07 SD unit, 95% CI: 0.81, 1.41) times higher odds of prevalent dementia and separately 1.05 (95% CI: 1.02, 1.08, FDR adjusted *p*-value = 0.009; OR = 1.28 SD unit, 95% CI: 1.12, 1.46) times higher odds of prevalent cognitive impairment non-dementia. An association between the DunedinPoAm clock and both dementia and cognitive impairment non-dementia was detected but became non-significant when fully adjusting for covariates (Table 3 & Supplemental Table S4).

3.5. GrimAge age acceleration mediates the effect of cytokines on cognitive impairment

In fully adjusted mediation models, we observed that the GrimAge acceleration mediated part of the association between three different cytokines and cognitive impairment (dementia and cognitive impairment non-dementia combined). In a model with CRP as the primary exposure, the GrimAge acceleration mediated 22.6% of the total effect (95% CI: 4.3%, 97.1%), with an indirect effect OR of 1.016 (95% CI: 1.005, 1.025; Figure 1 & Supplemental Table S5). In models with IL-6 and IGF-1 as the primary exposures, the GrimAge acceleration mediated 17.7% (95% CI: 7.0%, 50.9%; indirect effect OR: 1.018, 95% CI: 1.006, 1.029) and 6.1% (95% CI: 1.5%, 19.3%; indirect effect OR: 0.996, 95% CI: 0.993, 0.999) of the total effects, respectively (Figure 1 & Supplemental Table S5). The observed mediation effects were small in magnitude and sensitive to potential unmeasured confounders. Our mediational e-values indicated that an unmeasured confounder associated with both CRP and cognitive impairment of an approximate magnitude of 1.14 could completely explain away the observed mediated effect (Supplemental Table S5). To provide further context, observed confounders in our direct effect model such as age (OR: 1.07, 95% CI: 1.05,1.08), race (Non-Hispanic Black, OR: 2.93, 95% CI = 2.25, 3.81; Hispanic, OR: 3.41, 95% CI = 2.61, 4.46), and education (High school or less, OR = 7.07, 95% CI = 3.94 to 14.1) (Supplemental Table S6) had larger odds ratios compared to the mediational E-value of 1.14. Mediational e-values of similar magnitude were observed for IL-6, and IGF-1 (Supplemental Table S5).

3.6. Sensitivity analysis

In sexstratified analyses, the overall patterns of association between cytokine levels, DNA methylation age acceleration, and cognitive impairment showed stronger statistical evidence among males than females (Supplemental Tables S7–S11). The association between CRP and dementia was significant only in males (OR: 1.29; 95% CI: 1.07–1.56; FDR = 0.05, OR: 1.03; 95% CI: 0.85–1.24 females). The association between CRP and GrimAge acceleration was significantly positive in both males (β = 0.63; 95% CI: 0.47–0.79) and females (β = 0.51; 95% CI: 0.39–0.63). Mediation proportions for CRP were 37% in females (indirect effect OR: 1.012, 95% CI: 0.999, 1.022) and 16% in males (indirect effect OR: 1.017, 95% CI: 1.001, 1.033), but the 95% CI for females crossed the null.

4. Discussion

In a large and diverse sample of older adults in the United States, we observed that higher circulating blood levels of pro-inflammatory cytokines and lower levels of anti-inflammatory cytokines were associated with cognitive impairment, and that these associations were partially mediated by accelerated biological aging (6.1%−22.6% mediation), as assessed using DNA methylation clocks. These findings suggest a connection between peripheral systemic inflammation in neurodegenerative disorders such as Alzheimer’s disease and other dementias [46]. Altogether, these findings suggest that accelerated DNA methylation aging may be an important cellular mechanism through which cytokines influence cognitive decline during aging.

Our results are consistent with other observational studies describing the association between blood-circulating cytokine levels and cognitive impairment [47–51]. In our sample, we found that higher blood levels of CRP and IL-6 were associated with greater odds of prevalent dementia and cognitive impairment non-dementia. Similarly, a case-cohort study (n = 727) within the Rotterdam cohort found that increased blood CRP levels were associated with a higher risk of dementia [47]. Also consistent with our findings, a longitudinal study with a representative sample of 3,075 African-American and White participants found that elevated CRP was associated with increased odds of cognitive decline over a 2-year follow-up period [48]. A meta-analysis of pro-inflammatory biomarkers and cognition in older adults found that high levels of IL-6 were associated with cognitive decline, but this study did not find the similar association with high CRP [49]. In contrast, high circulating levels of the anti-inflammatory cytokine IGF-1 are often associated with greater brain volume and a lower risk of dementia [50]. Indeed, in the Framingham Heart Study (n = 3,582), greater IGF-1 levels were associated with a lower risk of incident dementia [51]. Our cross-sectional results are consistent with these studies and further suggest that DNA methylation age acceleration may be a biological mechanism linking inflammatory cytokine levels with cognitive impairment.

Our results thus supported our hypothesis that accelerated DNA methylation aging is a plausible mechanism through which inflammatory cytokines influence cognitive status in older adults. DNA methylation clocks have previously been associated with age-related diseases and cognitive decline [14–16,52], suggesting that accelerated DNA methylation aging may be an important mechanism underlying progressive cognitive deterioration. Our study demonstrated that GrimAge acceleration was associated with worse cognitive status and may partially explain the relationship of CRP and IL-6 (pro-inflammatory cytokines), as well as IGF-1 (anti-inflammatory cytokine), with cognitive impairment. In addition to the GrimAge, the DunedinPoAm clock showed an association with dementia, and to a lesser extent with cognitive impairment non-dementia. However, these associations did not meet the threshold of FDR statistical significance. These two phenotypic clocks (GrimAge and DunedinPoAm) have been found to be more predictive of adverse health outcomes such as cognitive decline than the traditional chronological clocks [15,24,52]. For example, GrimAge integrates DNA methylation – based surrogates of several plasma proteins (e.g., GDF15, TIMP1, and PAI-1) that are well-established markers of inflammation and cellular senescence, along with smoking pack-years – a significant contributor to chronic inflammation and disease risk [15,24]. Therefore, GrimAge may serve as a more comprehensive indicator of biological aging than other DNA methylation clocks. Even though we were unable to examine the CpG-level methylation patterns of specific cytokine-related genes (e.g., IL6, TNF), mounting evidence underscores the role of age-associated DNA methylation changes as a cellular mechanism linking systemic inflammation to chronic and auto-immune diseases [53,54]. For instance, age-related changes in the innate and adaptative immune response are characterized by an overexpression of senescent T cell profiles in circulating blood. The overexpression of the T CD8+ phenotype is a highly regulated epigenetic process and may be induced by cytokines such as CRP and IL-6 [55,56]. A recent study exploring the trajectories of inflammatory biomarkers, immune cell profiles, and epigenetic aging in the Lothian Birth Cohort (n = 1,091)

demonstrated a cross-sectional association between high levels of each of CRP and IL-6 and age-accelerated Hannum residuals [56]. Our larger sample size provides enhanced power to detect cross-sectional associations between cytokines and both phenotypic and chronological aging measures compared to these previous studies. Future studies will be able to examine gene specific and pathway analyses. In addition, while we used peripheral blood-based biomarkers in this study, systemic inflammation can influence the central nervous system function through increased permeability of the blood–brain barrier and microglial priming [57–59]. Prior studies have demonstrated that elevated systemic levels of IL-6 and CRP are associated with microglial activation and neurodegeneration [57,60]. Thus, the cytokine and epigenetic signatures may reflect inflammaging processes that influence both peripheral and central pathways relevant to cognitive impairment. Future studies should incorporate cerebrospinal fluid biomarkers or neuroimaging to assess central nervous system inflammation.

Our analysis is limited by its cross-sectional design. Although our directed acyclic graph implies a temporal relationship between exposure (cytokine levels), mediator (DNA methylation age acceleration), and outcome (cognitive impairment), these variables were collected at the same time, and hence we are unable to rule out reverse causality. We also did not use cognitive data from 2018 because doing so would have substantially reduced the analytic sample size and potentially introduced survivor bias. In addition, we combine dementia and CIND to enhance the statistical power in our mediation models, but it may obscure clinically meaningful differences between these conditions. Future research should incorporate longitudinal assessments of inflammatory biomarkers, DNA methylation age acceleration, and cognitive outcomes in larger samples to allow for clinically specific analyses. Additionally, we were not able to explore the potential for race-dependent relationships between exposure to cytokines, DNA methylation age acceleration, and adverse cognition, because this study was limited to a subsample of the HRS with a small proportion of participants racialized as non-Hispanic Black or Hispanic. Our limited sample size hindered our ability to conduct stratify our analyses. Our analysis also has several strengths. To our knowledge, it is the first study to examine whether DNA methylation age acceleration, as captured by the GrimAge clock, mediates the relationship between systemic inflammation and cognitive impairment in a large, diverse sample of older adults. Our analytic sample was larger than that of most previous studies on this topic and included rich data on well-known confounding variables. Importantly, a strength of our study is that the HRS has provided clinically measured cell-type proportions (e.g., granulocytes, lymphocytes, monocytes). Cell type proportions are important precision variables because DNA methylation levels are influenced by tissue heterogeneity and immune cell composition, and we were able to adjust for cell-type proportions in our statistical models. Future studies may compare our findings to those with DNA methylation-derived estimates of additional leukocyte subsets, which were not available in our study. We also included APOE-ε4, a well-known genetic risk factor for dementia. Future studies with time-ordered components could shed light on the role of chronic conditions in the inflammatory response of aging adults and their contribution to cognitive impairment.

5. Conclusions

In conclusion, our study demonstrated a novel finding on the link between the inflammatory response, cellular processes associated with accelerated aging, and cognitive impairment among older adults. Through mediation analysis, we found that the effect of cytokines in cognitive impairment may be partially mediated through DNA methylation age acceleration. The interplay between persistent inflammatory levels and molecular mechanisms related to premature cognitive aging should further be explored. Future prospective and longitudinal studies should devote attention to understanding the temporal association between these physiological mechanisms to identify what other pathways influence cognitive impairment beyond the dominant amyloid hypothesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Disclosure statement

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Data availability statement

Demographic and cognitive measures are publicly available through the Health and Retirement Study (https://hrs.isr.umich.edu/). Cytokine measures, complete blood counts, and DNA methylation age data presented in this study were accessed from the http://hrs.isr.umich.edu/data-products data repository, Health Data: 2016 Biomarker Data release date November 2020 (Early v1.0). Health and Retirement Study genetic data are available through dbGaP (https://dbgap.ncbi.nlm.nih.gov; phs000428.v2. p2) or NIAGADS (niagads.org; NG00119 and NG00132. Code to perform the analyses is available (https://github.com/bakulskilab).

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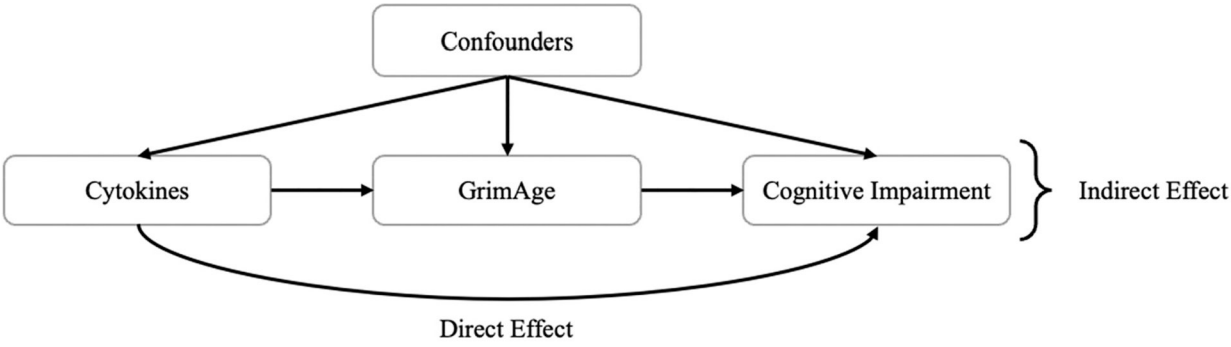
Article highlights

- Inflammation has been associated with cognitive impairment, and the potential linking role of DNA methylation age acceleration has not been explored.
- In a large sample of US adults ages 50 and above (n = 3,346), we estimated the associations between cytokines, DNA methylation age acceleration, and cognitive status.
- A doubling of the levels of the circulating cytokine interleukin 6 (IL-6) was associated with 12% higher odds of cognitive impairment and with older DNA methylation age acceleration by 0.77 years.
- The association between IL-6 and cognitive impairment was 17.7% mediated by DNA methylation age acceleration.
- We confirmed that inflammation was associated with cognitive impairment, and we offered new suggestive evidence that this is partially mediated through DNA methylation age acceleration.



Cytokine	Mediated (Proportion)	95% Confidence Interval
C-reactive protein	22.6%	(4.3%, 97.1%)
Interleukin-6	17.7%	(7.0%, 50.9%)
Insulin-Like Growth Factor 1	6.1%	(1.5%, 19.3%)

Figure 1.

Directed acyclic graph illustrating the hypothesized relationship between cytokines, GrimAge acceleration, and cognitive impairment.

Notes: Mediation estimates represent the proportion of the total effect of a cytokine on cognitive impairment that is due to DNA methylation age acceleration. The confounders include socio-demographic variables (age, sex, racialized social group, education), behavioral factors (body mass index, exercise, smoking status, alcohol consumption), chronic conditions, APOE- ε4 allele carrier status, and cell-type proportions (percent granulocytes and lymphocytes) in the Health and Retirement Study 2016 wave (n = 3,346).

Table 1.

Sample distribution by cognitive status in the Health and Retirement Study, wave 2016.

Characteristic[a]	Overall *n* = 3,346	Normal Cognition *n* = 2,678	Cognitive Impairment Non-Dementia *n* = 543	Dementia *n* = 125	*p*-value[b]
Cytokines					
C-Reactive protein (μg/mL)	2.30 (1.12,4.99)	2.24 (1.10,4.90)	2.38 (1.19,5.33)	2.99 (1.23,5.59)	0.141
Interleukin-10 (pg/mL)	3.44 (2.79,4.34)	3.40 (2.77,4.27)	3.73 (2.93,4.63)	3.58 (3.11,4.64)	0.317
Interleukin-1RA (pg/mL)	497.24 (370.86,690.83)	496.82 (368.42,691.12)	498.76 (380.75,689.90)	495.80 (362.56,679.31)	0.315
Interleukin-6 (pg/mL)	3.88 (2.57,6.32)	3.68 (2.48,5.92)	4.52 (2.99,7.25)	4.86 (3.13,8.26)	0.202
Insulin-Like Growth Factor-1 (ng/mL)	98.00 (75.00,124.00)	100.00 (77.00,126.00)	93.00 (69.00,117.00)	85.00 (58.00,113.00)	< 0.001
Transforming Growth Factor-β1 (ng/mL)	46.15 (37.55,55.40)	46.62 (38.22,56.19)	44.92 (35.71,53.27)	42.84 (35.35,52.05)	< 0.001
Tumor Necrosis Factor-R1 (ng/mL)	1.64 (1.33,2.09)	1.60 (1.32,2.02)	1.78 (1.38,2.35)	1.93 (1.47,2.53)	< 0.001
Age Acceleration (years)					
Phenotypic Clocks					
GrimAge	0.00 (4.76)	−0.27 (4.69)	1.13 (4.81)	1.00 (5.02)	< 0.001
DunedinPoAm	0.00 (6.44)	−0.23 (6.30)	0.85 (6.85)	1.16 (7.11)	< 0.001
Levine	0.00 (6.82)	−0.13 (6.78)	0.56 (6.91)	0.38 (7.27)	0.83
Chronologic Clocks					
Horvath	0.00 (6.52)	0.11 (6.47)	−0.57 (6.43)	0.13 (7.63)	0.081
Hannum	0.00 (5.31)	0.00 (5.29)	0.00 (5.26)	−0.03 (5.95)	0.998
HorvathSkin	0.00 (4.45)	0.01 (4.41)	0.00 (4.48)	−0.17 (5.35)	0.909
Age (years)	69.96 (9.63)	68.89 (9.18)	73.56 (9.94)	77.29 (10.62)	< 0.001
Race/ethnicity					< 0.001
Non-Hispanic Black	554 (17%)	387 (14%)	131 (24%)	36 (29%)	
Non-Hispanic White	2,340 (70%)	1981 (74%)	307 (57%)	52 (42%)	
Hispanic	452 (14%)	310 (12%)	105 (19%)	37 (30%)	
Sex					0.109
Female	1,957 (58%)	1,590 (59%)	300 (55%)	67 (54%)	
Male	1,389 (42%)	1,088 (41%)	243 (45%)	58 (46%)	
Educational Category					< 0.001
> College	312 (9.3%)	301 (11%)	9 (1.7%)	2 (1.6%)	
College/Some	707 (21%)	634 (24%)	69 (13%)	4 (3.2%)	

Characteristic[a]	Overall *n* = 3,346	Normal Cognition *n* = 2,678	Cognitive Impairment Non-Dementia *n* = 543	Dementia *n* = 125	*p*-value[b]
High school or <	2,327 (70%)	1,743 (65%)	465 (86%)	119 (95%)	
APOE-ε4					< 0.001
At least 1 copy	838 (25%)	631 (24%)	165 (30%)	42 (34%)	
No copy	2,508 (75%)	2,047 (76%)	378 (70%)	83 (66%)	
Body Mass Index (kg/m^2)	28.86 (6.23)	29.11 (6.32)	28.04 (5.71)	27.12 (5.66)	< 0.001
Smoking status					0.703
Current Smoker	361 (11%)	286 (11%)	60 (11%)	15 (12%)	
Former Smoker	1,505 (45%)	1,191 (44%)	255 (47%)	59 (47%)	
Never Smoker	1,480 (44%)	1,201 (45%)	228 (42%)	51 (41%)	
Exercise (>1 time per week)					< 0.001
Yes	2,378 (71%)	1,998 (75%)	320 (59%)	60 (48%)	
No	968 (29%)	680 (25%	65 (52%)	223 (41%)	
Number of drinks per day	0.81 (1.43)	0.89 (1.48)	0.52 (1.17)	0.34 (0.78)	< 0.001
Number of Chronic Conditions					< 0.001
≥3	1,510 (45%)	1,130 (42%)	304 (56%)	76 (61%)	
1–2	1,523 (46%)	1,275 (48%)	208 (38%)	40 (32%)	
None	313 (9.4%)	273 (10%)	31 (5.7%)	9 (7.2%)	
Cell Type Proportions (%)					
Granulocytes	61.72 (9.50)	61.61 (9.31)	62.03 (10.10)	62.86 (10.79)	0.254
Monocytes	8.57 (2.43)	8.55 (2.40)	8.66 (2.49)	8.60 (2.62)	0.614
Lymphocytes	29.75 (9.25)	29.88 (9.11)	29.36 (9.67)	28.59 (10.19)	0.175

Notes: APOE-ε4 = apolipoprotein E ε4 allele carrier status.

[a] Data presented as mean (standard deviation), median (25%,75%) or count (%) where applicable.

[b] One-way ANOVA.

Table 2.

Association for each doubling of cytokine levels with prevalent dementia and cognitive impairment non-dementia.

	Dementia versus normal cognition (*n* = 2,803)			Cognitive impairment non-dementia versus normal cognition (*n* = 3,221)		
Cytokines	**OR**	**[95% CI]**	**FDR**	**OR**	**[95% CI]**	**FDR**
C-Reactive protein[a]	1.16	[1.01,1.32]	0.093*	1.07	[1.00,1.15]	0.107*
Interleukin-6[a]	1.19	[1.00,1.41]	0.093*	1.12	[1.02,1.22]	0.084*
Interleukin-10[a]	1.06	[0.78,1.42]	0.8	1.04	[0.88,1.21]	0.8
Interleukin-1RA[a]	1.08	[0.79,1.46]	0.8	0.98	[0.84,1.14]	0.8
Insulin-Like Growth Factor-1[b]	0.88	[0.78,0.98]	0.093*	0.94	[0.89,1.00]	0.107*
Transforming Growth Factor-β1	1.00	[0.98,1.01]	0.8	1.00	[0.99,1.00]	0.42
Tumor Necrosis Factor-R1[a]	1.10	[0.77,1.52]	0.8	1.12	[0.94,1.33]	0.35

Notes: OR = odds ratio; CI = confidence interval in brackets; FDR = false discovery rate. The model was adjusted for race, age, sex, education categories, APOE-ε4 allele status, smoking status, alcohol consumption, body mass index, exercise, and chronic conditions.

[a]Variable log base 2 transformed.

[b]Squared root transformed.

*Indicates $p < 0.05$.

Table 3.

Association between DNA methylation age accelerated and prevalent dementia and cognitive impairment non-dementia.

	Dementia versus normal cognition (*n* = 2,803)			Cognitive impairment non-dementia versus normal cognition (*n* = 3,221)		
DNA methylation age acceleration (years)	**OR**	**[95% CI]**	**FDR**	**OR**	**[95% CI]**	**FDR**
Phenotypic Clocks						
GrimAge	1.01	0.96, 1.07	0.932	1.05	1.02, 1.08	0.002 *
DunedinPoAm	1.01	0.97, 1.04	0.932	1.01	0.99, 1.03	0.45
Levine	1.00	0.97, 1.03	0.932	1.01	0.99, 1.02	0.4
Chronologic Clocks						
Horvath	1.01	0.98, 1.04	0.932	0.99	0.97, 1.00	0.204
Hannum	1.02	0.98, 1.05	0.932	1.00	0.99, 1.02	0.72
HorvathSkin	1.00	0.96, 1.04	0.932	1.00	0.98, 1.02	0.9

Notes: OR = odds ratio; CI = confidence interval in brackets; FDR = false discovery rate.

* Indicates $p < 0.05$.

The model was adjusted for race, age, sex, education categories, APOE-ε4 allele status, cell components, smoking status, alcohol consumption, body mass index, exercise, and chronic condition.