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Trajectories of peripheral interleukin-6, structure of the hippocampus, and cognitive impairment over 14 years in older adults

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Abstract

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Conflicts of Interest: None.

We aimed to investigate if trajectory components (baseline level, slope and variability) of peripheral IL-6 over time were related to cognitive impairment and smaller hippocampal volume, and if hippocampal volume explained the associations between IL-6 and cognitive impairment. Multivariable regression models were used to test the association between IL-6 trajectory components with change in neuroimaging measures of the hippocampus, and with cognitive impairment among 135 older adults (70–79 years at baseline) from the Healthy Brain Project over 14 years. IL-6 variability was positively associated with cognitive impairment (OR = 5.86, 95% CI:1.24, 27.61) and with greater decrease per year of gray matter volume of the hippocampus (β= −0.008, SE=0.004, p=0.03). After adjustment for hippocampal volume, the odds ratio of cognitive impairment decreased for each unit of IL-6 variability, and confidence intervals widened (OR=4.36, 95% CI: 0.67, 28.29). Neither baseline levels nor slopes of IL-6 were related to cognitive impairment or hippocampal volume. We believe this has potential clinical and public health implications by suggesting adults with stable levels of peripheral IL-6 may be better targets for intervention studies for slowing or preventing cognitive decline.

Keywords

cognitive impairment; interleukin-6; hippocampal morphology; aging; epidemiology

1. Introduction

The relationship between peripheral interleukin-6 (IL-6), and risks of dementia, Alzheimer's disease (AD) or memory impairment have been widely investigated. Many studies indicated significant associations between elevated peripheral IL-6, usually measured at baseline, and risk of these cognitive outcomes.(Economos, et al., 2013; Elderkin-Thompson, Irwin, Hellemann, & Kumar, 2012; Grassi-Oliveira, Bauer, Pezzi, Teixeira, & Brietzke, 2011; Mooijaart, et al., 2013; Schram, et al., 2007; Yaffe, et al., 2003) However, such studies are limited by cross-sectional study design, or by having IL-6 measured at only one point in time, often many years prior to the measurement of study outcomes, in longitudinal studies. This is a limitation because IL-6 is variably expressed in a variety of biological situations, and has considerable intra-individual variability, thus it is important to quantify the variability of IL-6 at repeated time points, in addition to absolute values. However, prior studies did not characterize this variability over time, due to lack of serial IL-6 measurements.(Economos, et al., 2013; Elderkin-Thompson, et al., 2012; Mooijaart, et al., 2013; Yaffe, et al., 2003) Other fields have also started to investigate the importance of variability in peripheral cytokines, and found that variability is, in fact, an important predictor of disease outcomes like cardiovascular disease.(deGoma, et al., 2012; Koenig, et al., 2003)

While IL-6 is a pleiotropic humoral factor, it has been of particular interest given its pathologic effects in the setting of many chronic inflammatory diseases.(Hunter & Jones, 2015) In epidemiology of aging, IL-6 has become a prominent parameter that investigators measure given its reasonable associations with certain age-related diseases, disability, and mortality.(Singh & Newman, 2011) IL-6 has several biological properties that make it a reasonable biomarker. IL-6 decays less rapidly than other peripheral blood cytokines, thus

its peripheral measurements are considerably more reliable.(Gadient & Otten, 1994; Marsland, 2011; Schobitz, De Kloet, & Holsboer, 1994; Vitovic, et al., 2000) Peripheral IL-6 can pass the blood brain barrier, and stimulate central inflammatory response indirectly via the vagal nerve.(Banks & Kastin, 1991; Maier & Watkins, 1998; Tracey, 2002) IL-6 receptors are distributed throughout the brain with a particularly high concentration in the hippocampus and prefrontal cortex. (Hampel, et al., 2005) IL-6 may modulate cognitive function through IL-6-mediated signaling in the brain.(Gadient & Otten, 1994; Marsland, 2011; Schobitz, et al., 1994; Vitovic, et al., 2000) All these properties of IL-6 provide a basis for its usefulness as a peripheral marker of brain function.

In cross sectional studies, an association between elevated peripheral IL-6, hippocampal volume, and lower executive function and performance in middle-aged adults has been reported.(Kesler, et al., 2013; Marsland, Gianaros, Abramowitch, Manuck, & Hariri, 2008) Peripheral IL-6 has also been associated with magnetic resonance imaging (MRI) detected abnormalities, which is found not only in those with cognitive impairment or AD, but also among those with major depressive disorder and schizophrenia.(Frodl & Amico, 2014; Savitz, et al., 2013) There have been additional functional MRI studies showing cross-sectional associations between peripheral IL-6, and the levels of activation in the hippocampus and medial temporal lobe.(Gianaros, et al., 2014; Harrison, Cercignani, Voon, & Critchley, 2014; Harrison, Doeller, Voon, Burgess, & Critchley, 2014) Collectively, prior studies strongly indicate a link between circulating IL-6 levels, and cognitive decline, likely through changes in the hippocampus, an area known to regulate memory formation. However, longitudinal studies are needed to investigate whether changes in IL-6 levels over time are associated with micro- or macro- structural brain changes and subsequent cognitive outcomes. Therefore, we undertook the present study to investigate the association between trajectories (baseline level, slope and variability) of peripheral IL-6, micro and macro neuroimaging markers of hippocampal integrity, and clinically adjudicated cognitive impairment over a period of 14 years in the Health Aging and Body Composition (Health ABC) study. We sought to determine whether the association between IL-6 trajectories and cognitive impairment was mediated by neuroimaging markers of hippocampal integrity. We predicted that higher and sustained levels of peripheral IL-6 would be related to cognitive impairment and smaller hippocampal volume, and that hippocampal volume would explain the associations between IL-6 and cognitive impairment.

2. Material and Methods

2.1 Study population

Community-dwelling white and black older adults were enrolled in the ongoing Health ABC study, a prospective cohort study which began in 1997. At baseline, adults ranged in age from 70 to 79 years, and lived in Memphis, TN or Pittsburgh, PA. Participants were recruited from a random sample of Medicare eligible adults living within designated zip codes, and were eligible if they reported no difficulties performing activities of daily living, walking a quarter mile, or climbing 10 steps without resting. They were also free of life-threatening cancers, and planned to remain within the study area for at least three years. This study was approved by the institutional review boards of the University of Pittsburgh, the

University of Tennessee, Memphis, and of the Coordinating Center, the University of California San Francisco. All participants signed a written informed consent.

Participants at the Pittsburgh study site were asked to participate in the Healthy Brain Project (HBP), a neuroimaging substudy of cognition and mobility in 2006–07 (Year 10 of the parent Health ABC cohort). These participants have been seen at regular intervals at the Pittsburgh site from 1997–98 to 2011–12. Of the 1,527 participants enrolled in the study in 1997–98 at the Pittsburgh site, 819 were alive, and were contacted in 2006–07 to participate in the HBP. Among these, 315 received a brain MRI at 3 Tesla. Among the 315 participants with a 3 Tesla, 3 did not have complete data on IL-6, and 63 died before cognitive adjudication was completed and did not have cognitive status adjudicated. Among the 249 participants with complete data on MRI, IL-6 and cognitive adjudication, all had a Modified Mini-Mental Status Exam (3MS) scores ≥84 at the time of enrollment in the Healthy Brain Project and the first MRI; as a 3MS score ≤80 usually signifies dementia, all participants were considered free of dementia at time of the first MRI.(Metti, et al., 2014; Slinin, et al., 2010) Among the 249 with complete data of first MRI and cognitive status, 135 participants also received a second brain MRI in 2010–11 (Year 14), and all longitudinal analyses including 2010–11 outcomes or mediators pertain to the 135 with complete data on MRI at both time points. The 135 participants who survived to the second MRI had a higher mean BMI (27.8±4.5 vs. 26.4±4.6, p=0.02), and were slightly less likely to have a history of myocardial infarction (6.1% vs. 12.6%, p=0.06), when compared to those who only survived to the first MRI; these two groups did not differ significantly on any other demographic or medical characteristics.

2.2 Cognitive Impairment Outcome

Cognitive status was clinically adjudicated on Health ABC participants who were seen at the Year 14 site visit in 2010–11, using all data from previous visits as well as cognitive assessments at the time of the second MRI. Specifically, an extensive battery of cognitive tests was administered, concurrent with brain MRI and assessment of neurological function. The neuropsychological testing included (a) Premorbid intelligence: the American version of the National Reading test (AMNART) and Raven's Colored Progressive Matrices [Raven #192]; (b) Memory: California Verbal Learning Test (CVLT), and Rey-Osterreith figure; (c) Language: Boston Naming test and Verbal fluency test; (d) Visuoperceptual/ visuoconstructional: Block design and copy of a geometric figure; and (e) Executive Function: Stroop test.

This neuropsychological battery was designed following extensive consultation with neurologists and dementia experts. It was based on two goals: 1) identifying deficits in specific cognitive domains that characterize mild cognitive impairment (MCI) and its subgroups, and 2) diagnosing dementia. This battery was sensitive to cognitive impairment, and detailed normative data have previously been obtained through the Cardiovascular Health Study (CHS) Dementia study.(Lopez, et al., 2003) Adjudicated outcomes for the 249 participants included: cognitively normal (n=99), MCI (n=83), dementia (n=62), or no neurological data (n=5). Adjudicated outcomes for the 135 participants included: cognitively normal (n=57), MCI (n=57), dementia (n=19), or no neurological data (n=2). For the

purposes of this study, we created an outcome variable of cognitive impairment, which included those with both MCI and dementia, versus no cognitive impairment.

We also created a time-to-event variable to estimate onset of cognitive impairment, between study entry in 1997–99 (baseline) and adjudication of cognitive status in 2010–12 (year 14). The date of the first available record of dementia or cognitive impairment was identified by any of the following criteria: (a) First record of prescribed medication indicated for dementia (galantamine, rivastigmine, memantine, donepezil, or tacrine), collected at annual study visits with a drug inventory; (b) First hospital admission record indicating a dementia related event (for either a primary or secondary diagnosis of dementia related to admission), collected every six months; (c) A 3MS score of 90 or below (completed at baseline and years 3, 5, 8, 10, and 11), indicating cognitive impairment; or a race stratified decline of at least 1.5 standard deviations on the 3MS from baseline to the last available visit.(Kaup, et al., 2013; Yaffe, et al., 2013)

2.3 Interleukin-6

Measures of IL-6 were obtained from frozen serum or plasma collected six times throughout the study; at baseline (1997–99 or Year 1 of the study), and at Years 2, 4, 6, 8 and 10 (2006–07 when first MRI was collected). Samples were collected at study visits between 7 am and 9 am, after an overnight fast, frozen at −70°C and shipped to the Core Laboratory at the University of Vermont.(Yaffe, et al., 2003) Baseline serum IL-6 was measured by ELISA kits (R&D Systems, Minneapolis, MN). In Years 2, 4, 6, 8 and 10 serum IL-6 was measured at Wake Forest University using the same high sensitivity sandwich ELISA kit (R&D Systems). The inter-assay coefficients of variation for this assay were 14%, 11%, and 13% for low, medium and high ranges, respectively. To minimize inherent inter-laboratory differences, values were calibrated. Calibration was performed based on a set of 150 blind duplicate measurements obtained from each lab at year 1. A second calibration was performed on samples at year 8 to account for difference in sample source. This calibration was based on a set of 137 samples at year 6 that were derived from both serum and cell pack.

To allow for interpretation on a relative scale, and account for skewed distributions, IL-6 data were log transformed (natural log). Because a minimum of three measurements are required to define variability, all participants in the analytic cohort had IL-6 available at three to six time points for analysis of trajectories. To determine IL-6 trajectory slope and variability around the trajectory of IL-6, we used linear regression to model IL-6 over time for each person. The slope on the log scale can be interpreted as the annual relative change in the original non-transformed scale. The variability around the trajectory was calculated by determining the root mean square error (RMSE) in a model with a linear trajectory and analyzed by deciles. For slope, decile 10 was defined as steep slope, and deciles 1 through 9 were defined as non-steep slope; for variability, deciles 1–7 were defined as minimal variability, deciles 8–9 were moderate variability, and decile 10 was defined extreme variability. These analytic techniques were based on two previous studies examining trajectories of biological markers (Dehydroepiandrosterone Sulfate and inflammatory

markers) with outcomes of mortality and incident cognitive decline.(Cappola, et al., 2009; Metti, et al., 2014)

2.4 Neuroimaging

A 3T Siemens Tim Trio MR scanner with a Siemens 12-channel head coil was used for obtaining MRI scans in 2006–07 and 2010–11. Neuroimaging markers of the hippocampus were measured using MRI at 3 Tesla at both time points. Measures included mean diffusivity (MD) of the hippocampus and gray matter volume (GMV) of the hippocampus in 2006–07. In 2010–11, both of these measures were repeated, and percent change per year of MD and GMV were calculated. Image acquisition and analysis for this study has been previously described.(Rosano, et al., 2012) Briefly, magnetization-prepared rapid gradient echo (MPRAGE) images were acquired to obtain GM volumes of the hippocampus, and used in the processing of DTI data. Diffusion tensor images were acquired using single-short spin-echo sequence with 12 directions and preprocessed using the FMRIB's Diffusion Toolbox (Smith, et al., 2004) to remove unwanted distortions (voxel size = 2 mm × 2 mm; slice thickness = 3 mm). Using the segmentation of GM from the MPRAGE, the MD was restricted to GM of the hippocampus, as defined in the Automated Anatomical Labeling Atlas.(Tzourio-Mazoyer, et al., 2002)

2.5 Covariates

At baseline, demographic data including self-reported participant age, race, sex and education were recorded. Current smoking status was self-reported as current, ever or never. Disease algorithms indicating baseline prevalent, as well as incident over the course of the study, based on both self-report and physician diagnoses, recorded medications and laboratory data were used to create comorbidity variables indicating presence of diabetes mellitus, hypertension, stroke or transient ischemic attack (TIA), and myocardial infarction (MI) or coronary heart disease over the study period, including times when inflammatory markers were collected. Body mass index (BMI) (kg/m^2) was calculated from direct height and weight measurements at baseline. The Center for Epidemiologic Studies Depression Scale (CES-D) was used to assess depressive symptoms with a score ≥16 consistent with possible depression.(Radloff, 1977) Apolipoprotein E (*APOE*) genotype was determined using standard Single Nucleotide Polymorphism (SNP) genotyping techniques and dichotomized into having one or more *APOE* e4 alleles versus no allele(Hixson & Vernier, 1990) Creatinine and cystatin-C were obtained from frozen serum collected at baseline after an overnight fast. Samples were frozen at −70°C and were shipped to the Core Laboratory at the University of Vermont. Similarly, fasting glucose (mg/dL) was obtained from frozen serum, and total cholesterol (mg/dL) from frozen plasma collected at baseline after an overnight fast. An inventory of prescription and over-the-counter medications was recorded at baseline by examining participants' medication bottle(s). Consistent with previous studies using anti-inflammatory medication, we coded medications according to the Iowa Drug Information System (IDIS) code.(Pahor, et al., 1994; Yaffe, et al., 2003) With use of the IDIS, the daily use of anti-inflammatory drugs (IDIS code 2808), statins (IDIS code 2406), and oral estrogens (with or without progestins) (IDIS code 6816) was compiled.(Pahor, et al., 1994; Yaffe, et al., 2003)

2.6 Statistical analysis

Descriptive statistics were utilized to give a summary of the data. For continuous variables, means and standard deviations (or medians for non-normal variables) were calculated across time points, while sample proportions were calculated for categorical variables. To determine the bivariate association between baseline characteristics and the predictors, outcome and potential mediators, we conducted linear and logistic regression, where appropriate. Normality and fit assumptions were checked and met for all models.

Slope and variability of both IL-6 and CRP were modeled as time-varying covariates over the entire 10 years; at each measurement of either marker, slope and variability were recalculated using all measures through that date and carried forward until the next measurement of the marker. By updating the slope and variability at each measurement, the models were estimated with respect to current, not future, exposure status. Logistic regression models were performed to estimate the odds of cognitive impairment with predictors of baseline level of IL-6, most recent level of IL-6, trajectory slope, and variability around the trajectory entered into models individually, and then in combination. Linear regression was used to estimate the association between trajectory components of IL-6 and neuroimaging markers of the hippocampus. Finally, logistic regression was conducted to assess if percent changes in neuroimaging markers of the hippocampus mediated the association between IL-6 and incident dementia. All models were first assessed unadjusted, and then adjusted for factors that were significantly associated with IL-6 in bivariate models ($p \leq 0.05$), or known to be associated with our outcome of cognitive impairment (adjusted for age, race, sex, education and fasting glucose). We also further adjusted a model for *APOE* e4 status, and anti-inflammatory drugs to see how these variables affected our associations. All analyses were performed with SAS, version 9.4 (SAS Institute Inc., Cary, NC).

2.7 Sensitivity Analyses

Although there were no significant bivariate associations between prevalent and incident CHD, stroke/TIA, diabetes mellitus, or hypertension, we were concerned that health status over time would have a large affect on inflammation. Thus we conducted sensitivity analyses where we additionally adjusted all logistic models for these variables individually, and collectively. We further assessed if interim hospitalizations over 11 years affected these associations. Finally, we attempted to increase the power of our study by assessing the opposite association to see if minimally variable IL-6 levels were associated with decreased odds of cognitive impairment.

3. Results

The correlation coefficient of ln(IL-6) at baseline and at Year 10 was 0.47 ($p<0.001$). Between baseline and Year 2, 12 (8.9%) participants had a steep IL-6 slope versus 123 (91.1%) without; between Year 8 and Year 10, these numbers were similar with 15 (11.1%) have a steep slope versus 120 (88.9%) without a steep slope. These were not the same participants at both time points; only 1 participant had a steep IL-6 slope at all time points. When examining variability over the first 3 time points, 15 (12.7%) had extreme IL-6

variability (i.e. decile 10), and when examining the last 3 time points, 11 (8.2%) had extreme variability; only 6 had extreme IL-6 variability the entire course of the study. Steep IL-6 slope and extreme IL-6 variability were not correlated at first measurements (r=0.15, p=0.10) or at last measurements (r=0.10, p=0.26). There was no systematic increase or decrease of IL-6 over time.

At baseline (Year 1), participants were 72.6 (2.4) years of age, 82 (60.7%) were female, and 78 (57.8%) were white (Table 1). Being white and having higher education was associated with lower probability of having cognitive impairment (Table 1). Time-to-event for cognitive impairment was an average of 13.3 (±1.5) years for participants, indicating that on average, participants with cognitive impairment first showed signs of this impairment between the first and second MRIs, and less than 1 year before the cognitive adjudication.

Extreme IL-6 variability was related to greater odds of cognitive impairment in models adjusted for age, race, sex, education, fasting glucose, and *APOE* e4 (OR=5.86, 95% CI: 1.24, 27.61). Adding anti-inflammatory medications to the model did not significantly change the strength or direction of the association (OR=3.72, 95% CI: 0.99, 13.95). Associations of baseline IL-6, slope of IL-6 or IL-6 at time of first MRI with cognitive impairment were all non-significant (Supplemental Table 1).

Extreme IL-6 variability was also related to greater percentage decrease per year of gray matter volume of the hippocampus (β= −0.008, p=0.03), whereas associations with mean diffusivity of the hippocampus were not significant (Table 2). Percent change per year of gray matter volume of the hippocampus attenuated the association between IL-6 variability and cognitive impairment, as the odds ratio decreased and confidence intervals widened when it was added to the model adjusted for age, race, sex, education, fasting blood glucose and *APOE* e4 allele status (OR=4.36, 95% CI: 0.67, 28.29) (Table 3). Sensitivity analyses conducted showed prevalent and incident diseases over the study period did not affect this association (Supplemental Table 2). Similarly, hospitalizations over the course of the study period did not affect this association (data not shown). Minimally variable IL-6 levels also significantly predicted reduced odds of cognitive impairment (OR=0.17, 95% CI: 0.04, 0.81), after adjustment for age, race, sex, education, fasting blood glucose and *APOE* e4 allele status.

4. Discussion

In this longitudinal study of community-dwelling older adults, we found those with highly variable IL-6 were more likely to have faster decline in gray matter volume of the hippocampus during subsequent years, and were at a higher risk for a diagnosis of cognitive impairment. The relationship between highly variable IL-6 and cognitive status was modified by decreases in gray matter volume of the hippocampus. These are novel findings because they leverage repeated measurements of IL-6, changes in brain structure, and adjudicated cognitive outcomes instead of the usual one-time point measurement typical of cross sectional studies. Our findings expand on a multitude of work showing a relationship between high IL-6 level measured at one or two time points and increased risk of dementia or cognitive impairment.(Jenny, et al., 2012; Kravitz, Corrada, & Kawas, 2009; Schmidt, et

al., 2002; Yaffe, et al., 2003) First, we are looking at IL-6 measured between 3 and 6 time points over the course of a decade. Second, we are looking at several specific trajectory components of IL-6 to better understand which component is specifically affecting cognitive function. Third, we are investigating whether hippocampal structure mediates such a relationship, which allows us to gain a better understanding of the nature of a relationship between systemic IL-6 and cognitive impairment.

There are several potential mechanisms that could explain the association between IL-6 variability, decreased hippocampal gray matter volume, and cognitive impairment. First, animal models have suggested IL-6 activation may affect hippocampal gray matter volume by inhibiting neurogenesis and decreasing synaptic plasticity.(Marsland, et al., 2008) Second, single nucleotide polymorphisms (SNPs) of IL-6 genes could also contribute to the association, provided such SNPs would be those that directly affect IL-6 production, or those that alter the affinity of IL-6 to its receptor. Cross-sectional association between IL-6 SNPs, peripheral IL-6 levels and hippocampal volume have been reported for both healthy adults and schizophrenic patients.(Baune, et al., 2012; Kalmady, et al., 2014) Specifically, rs1800795 has been associated with reduced hippocampal volume in both patients with schizophrenia and in healthy older adults, and rs1800796 and rs1524107 have been associated with reduced risk of late onset AD; these IL-6 genotypes are worth exploring in future studies.(Baune, et al., 2012; Chen, et al., 2012; Kalmady, et al., 2014)

A third potential mechanism could be greater overall morbidity, especially in terms of vascular and metabolic disease, which we may be capturing when looking at IL-6 variability. Vascular disease risk factors such as diabetes, APOE gene variants, and metabolic syndrome components have all been associated with increased risk for cognitive decline.(Kivipelto, et al., 2001; Shah, et al., 2012; Yaffe, et al., 2004; Yaffe, Weston, Blackwell, & Krueger, 2009) However, we did not find these variables modified the observed association.

A fourth possibility is we could be capturing episodic illness or more frequent health events in older adults with highly variable IL-6, since peripheral IL-6 can be elevated in many disorders (i.e. liver disease, cancer, vascular disease, rheumatoid arthritis, etc.). We tested for this scenario by looking to see if interim hospitalizations explained the association between IL-6 variability and cognitive impairment; the point estimate and confidence intervals for the odds ratio of variability predicting cognitive impairment did not significantly change when interim hospitalizations over 11 years were added to the model. Perhaps subclinical illness or illness not severe enough to cause hospitalization would have a greater effect, but we did not have data to assess this possibility. We did conduct sensitivity analyses to determine if prevalent and incident CHD, hypertension, diabetes and stroke affected the associations, and they did not. This evidence supports the theory that the association is at least not fully caused by health status. Finally, we looked to see if the opposite association held true, in an attempt to increase the strength of our conclusions. We found that minimally variable IL-6 over time was associated with lower odds of cognitive impairment, thus strengthening our conclusions.

In this study, we cannot rule out that decreases in hippocampal volume are causing an increase in peripheral IL-6 or in IL-6 variability, especially since dementia has such a long period of underlying pathological processes that occur before overt symptoms or testing can indicate impairment. However, by including only those with 3MS scores >80 and by looking at time-to-event for cognitive impairment to estimate when onset of symptoms first started, our interpretation of the results is a valid possibility. We also cannot rule out that IL-6 variability may simply be a surrogate of other biological factors or events in old age, such as differential response to infection or stress. More research will be needed in the future to tease out the timing and nature of these highly complex relationships.

One interesting finding is that our previous analysis of the parent cohort did not find an association between high IL-6 variability and cognitive decline measured over a similar time period of 10 years.(Metti, et al., 2014) This discrepancy could be attributed to longer follow-up times employed in this study both for IL-6 measurements and cognitive measures, the application of a formal adjudication method for cognitive status, and the selection of a healthier cohort because of those who were eligible for and survived to MRI study dates. Future studies with longer follow-up and larger sample size of older adults with a wider range of comorbid conditions are warranted to differentiate the impact of IL-6 on cognitive status.

Strengths of this study include measurement of IL-6 between 3 and 6 time points, and MRIs conducted at two time points. All participants in this study were free of dementia, based on cognitive test scores at the time of the first MRI, and IL-6 was measured in years prior to the outcomes of cognitive status and brain structure, so while causality cannot be inferred, temporality of the relationship is established. We also have clinically adjudicated cognitive impairment on a sample of a large cohort that has been well characterized in terms of comorbidities and demographic data over 14 years, allowing us to investigate a large number of potential covariates and confounders.

When interpreting these results, several limitations may also be taken into consideration. The older adults in the Health ABC study are community-dwelling and free from overt physical disability at baseline, who all survived a minimum of 14 years post-baseline, and on average longer in order to obtain 2 MRIs and cognitive adjudication. Hence, the results are likely not generalizable to nursing home populations. Those lost to death or comorbidity before the end of the study could potentially bias our results. However, we believe this bias would result in underestimation of our association for several reasons. First, dementia itself can be fatal, so we likely have a smaller number of dementia cases in our analytic sample. Second, other diseases that could lead to study attrition and/or death could also be associated with IL-6 variability. Third, those with the most severe disease and severe inflammatory states were likely those lost to our analytic sample. Fourth, 14 years into the study, there was only a small sample available for MRI, and some of our wide confidence intervals reflect this small sample size. Fifth, we only investigated IL-6, so it is a relatively limited view of the physiologic humoral environment. One study, has demonstrated a wide array of cytokines including IL-6, and other humoral factors differ between functionally impaired and unimpaired older community dwelling adults.(Vallejo, et al., 2011) Therefore, measurement of a full panel of humoral markers over time may provide more useful

information. Nevertheless, we specifically chose IL-6 because of its ability to reach the brain, and a documented cross sectional association between IL-6 and hippocampal atrophy. Lastly, we found widespread use of anti-inflammatory medications. Our adjustment for daily use of these drugs did not affect the observed association between IL-6 variability and brain imaging characteristics, but information on intermittent use was not collected for this study.

4.1 Conclusions

We found highly variable IL-6 over 14 years was associated with cognitive impairment. This association was partially mediated by decreased hippocampal gray matter volume, which provides some evidence of a temporal relationship between variability in peripheral IL-6 and cognitive impairment or dementia. This result could have clinical and public health implications because it suggests that adults with stable levels of peripheral IL-6 may have less structural brain damage. If future studies validate these results, peripheral IL-6 is a potentially useful laboratory marker to obtain for older adults being seen and followed in memory clinics, as it may serve as a non-invasive indicator of disease progression and response to treatment. Future studies should further investigate the causal nature of these complex relationships, to better understand if highly variable peripheral IL-6 is leading to the hippocampal volume changes, or is a result of cell death in the hippocampus. Future studies should also examine these associations in larger populations of older adults, including more heterogeneous populations to determine if these findings extend beyond relatively healthy, community-dwelling older adults.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We used a prospective study to examine the association between trajectories of IL-6, cognitive impairment, and hippocampal volume.
- Extreme variability in IL-6 over time was associated with an increased risk of cognitive impairment.
- Extreme variability in IL-6 over time was associated with greater decrease per year of gray matter volume of the hippocampus.
- The association between IL-6 variability and cognitive impairment was weakened when hippocampal volume was added to the model.
- There were no significant associations between baseline level of IL-6 or slope of IL-6 and cognitive impairment or hippocampal volume.

Table 1

Population characteristics at baseline (1997–98), and associations with presence of cognitive impairment (dementia + MCI), adjudicated using data from 1997–99 to 2010–11.

Population Characteristic	Mean (sd), Median or N(%)	Cognitive Impairment
N	135	135
Age	72.6 (2.4)	β= −0.01, p=0.87
Female	82 (60.7%)	β= −0.27, p=0.44
White	78 (57.8%)	β= −1.00, p=0.006
Education ≤ HS	69 (51.1%)	β=0.75, p=0.03
Serum Creatinine (mg/dL)	1.0 (0.2)	β=0.27, p=0.79
Cystatin-C (mg/L)	1.1 (0.2)	β= −1.76, p=0.06
Fasting serum cholesterol (mg/dL)	212.4 (36.4)	β=0.001, p=0.78
Fasting Glucose (mg/dL)	93.0	β=0.003, p=0.65
Diabetes	28 (20.7%)	β= −0.58, p=0.19
Prevalent and Incident Diabetes*	53 (39.3%)	β= −0.23, p=0.51
History of Stroke/TIA	8 (5.9%)	β=0.55, p=0.47
Prevalent and Incident Stroke/TIA*	12 (8.9%)	β=0.92, p=0.18
History of Hypertension	80 (59.3%)	β= −0.35, p=0.40
Prevalent and Incident Hypertension*	126 (93.3%)	β= −0.47, p=0.52
History of Myocardial Infarction	17 (12.6%)	β= −0.14, p=0.79
Prevalent and Incident Coronary Heart Disease*	45 (33.3%)	β=0.37, p=0.33
Body Mass Index (kg/m^2)	27.8 (4.5)	β=0.05, p=0.28
CES-D Score (Max 60, ≥16)	4.2 (4.3)	β=0.01, p=0.76
Current smoker	5 (3.7%)	β= −0.42, p=0.65
Anti-inflammatory Medications	63 (46.7%)	β=0.22, p=0.57
APOE e4 positive status	32 (23.7%)	β=0.48, p=0.26

* Prevalent and incident variables calculated based on data from every study visit available over 14-year study period.

Table 2

Univariate association between IL-6 variability from 1997–99 to 2006–08 and changes in neuroimaging markers of the hippocampus from 2006–08 to 2010–11 in N=135 participants.

Variability Level	Gray matter volume, % change/year		Mean diffusivity, % change/year	
	β	p-value	Β	p-value
Minimal	Reference	-	-	-
Moderate	0.001	0.64	0.002	0.40
Extreme	−0.008	0.03	0.001	0.79

Table 3

Associations between IL-6 variability from 1997–99 to 2006–08 and cognitive impairment (dementia + MCI) adjudicated using data from 1997–99 to 2010–11 in 135 participants. Changes in coefficients from Model 2 to model 1 are used to assess mediation by gray matter volume, % change per year, measured from 2006–08 through 2010–11.

	OR (95% CI)	
Variability Level	**Model 1[a]**	**Model 2: further adjusted for gray matter volume, % change per year**
Minimal	Reference	-
Moderate	0.88 (0.42, 1.88)	1.24 (0.41, 3.76)
Extreme	5.86 (1.24, 27.61)	4.36 (0.67, 28.29)

[a]Model 1 adjusted for age, race, sex, education, fasting glucose and *APOE* e4 status.