Alzheimer's disease neuropathology, inflammation and epigenetic age: do sex and APOE matter?

Paula Duarte-Guterman[1*], Amy M. Inkster[2,3*], Arianne Y. Albert[4], Cindy K. Barha[5], Wendy P. Robinson[2,3], Liisa A.M. Galea[1], on behalf of the Alzheimer's Disease Neuroimaging Initiative[δ]

1. Djavad Mowafaghian Centre for Brain Health and Department of Psychology, University of British Columbia, Vancouver, BC, Canada
2. Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada.
3. BC Children's Hospital Research Institute, Vancouver, BC, Canada
4. Women's Health Research Institute of British Columbia, Vancouver, BC, Canada
5. Djavad Mowafaghian Centre for Brain Health and Department of Physical Therapy, University of British Columbia, Vancouver, BC, Canada

Address all correspondence and requests for reprints to:
L. A. M. Galea, PhD
Djavad Mowafaghian Centre for Brain Health
2215 Wesbrook Mall
Vancouver, British Columbia
V6T 1Z3, Canada
E-mail: lgalea@psych.ubc.ca.

* Considered co-first authors.

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ABSTRACT

Alzheimer's disease (AD) disproportionately affects females and the progression from cognitively normal to mild cognitive impairment (MCI) and to AD may be sex-specific. Using the ADNI database, we analysed the effect of sex and APOE genotype and sex and diagnosis (cognitively normal (CN), MCI, AD) on: AD-related endpoints (cognition, hippocampal volume, amyloid beta, tau-pathology), immune, neuroplasticity, and stress markers, and blood epigenetic age. More males were diagnosed with MCI but there was no sex difference in those diagnosed with AD, suggesting the progression from CN, MCI to AD may be sex-specific. Several biomarkers may influence sex differences in incidence and progression of MCI and AD. We found that females had higher levels of plasma CRP and lower levels of CSF IL-8, IL-16, IgA, and ICAM1 than males. Increasing number of APOE ε4 alleles and diagnosis also decreased plasma CRP in both sexes. Sex, diagnosis, or APOE genotype did not affect epigenetic age acceleration. We found, consistent with other studies, that females had higher levels of CSF tau-pathology that was disproportionately affected by APOE genotype compared to males. Females also had better memory (including verbal) scores than males, which may suggest a delay in the onset of cognitive decline or diagnosis. Sex differences in immune biomarkers and hippocampal volume (direction was dependent on method of correction) indicate that the underlying physiology during aging may be sex-dependent.

Keywords: Sex differences; Alzheimer's disease; APOE genotype; inflammation; neuroplasticity; epigenetic age; hippocampus; memory.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease characterized by severe cognitive decline [1]. Modifiable risk factors associated with AD include stress [2], sociocultural or lifestyle factors (e.g., education, marital status, exercise), and medical conditions [diabetes, obesity, and cardiovascular disease; ,3–5]. Non-modifiable risk factors include age, biological sex, and APOE genotype [6]. In some studies, females show greater neuropathology (brain atrophy, neurofibrillary tangles) and cognitive decline than males which depends on whether females are already cognitively impaired (mild cognitive impairment (MCI) or AD) and also on APOE genotype, a strong genetic risk factor for AD [7–14].

The hippocampus is one of the first brain areas to show atrophy with AD [15–17] and hippocampal atrophy correlates with cognitive decline [18] and AD pathology [neurofibrillary tangles; ,19]. Previous studies using the Alzheimer's Disease Neuroimaging Initiative (ADNI) indicate that females with AD have greater brain atrophy rates and cognitive decline than males with AD [9,10,13]. However, there is limited research into the role of sex and interactions with APOE ε4, a well-known genetic risk factor of AD [20], in the possible mechanisms underlying AD. In females between 65 and 75 years, one allele of APOE ε4 increases the risk of AD by 4-fold relative to males, indicating that the APOE genotype affects males and females differently [21]. Understanding why females are at a higher risk and have a higher burden of the disease is important for the development of tailored treatments based on sex and genetics.

Using the ADNI database, studies have examined the interaction of APOE genotype and biological sex on cognitive function and AD biomarkers but findings are not consistent. For example, some studies using ADNI have found a stronger association between APOEε4 and CSF tau levels in females compared to males [22] but this is not found in all ADNI studies [23,24]. Differences in type of statistical analyses, sample size, and how many groups were included in

the analyses (cognitively healthy, MCI, AD), are likely reasons for the discrepancies between studies. In addition, some studies have focused only on one aspect of AD, for example some studies have investigated how sex and APOE genotype affect either cognition/hippocampal volumes [14,25–27] or AD biomarkers [23,28]. Aging and AD are multifaceted processes, therefore one objective of our study is to employ a multivariate approach examining how sex, APOE genotype and diagnosis affect physiological and aging markers, AD biomarkers, hippocampal volumes and cognitive endpoints.

Chronic inflammation is a hallmark of AD, as evidenced by increased expression of proinflammatory cytokines in the brains of AD patients without regard to sex, which can exacerbate AD pathology [29–31]. There is however increasing evidence that there are sex differences in immune responses with females mounting a stronger response compared to males after an acute challenge [32,33]. In response to an endotoxin, females had higher levels of proinflammatory plasma cytokines (TNF-α and IL-6) while males had higher levels of anti-inflammatory plasma IL-10 [32,34]. Systemic inflammation, indicated by levels of C-reactive protein (CRP), is higher in females than in males [35]. Higher systemic inflammation (including plasma CRP) in midlife are also associated with higher brain amyloid levels later in life in healthy males but not females [36], 2018) and with cognitive decline later in life in both sexes [37]. Sex differences in the immune and inflammatory systems may differentially affect neuroplasticity [38,39]. In addition, peripheral cortisol, the main stress hormone in humans, is elevated in AD [40] and is associated with higher amyloid levels in the brain [41], a reduction in hippocampal volume and negative effects on cognition in older individuals [42] that may depend on MCI status [43]. Peripheral cortisol is also associated with elevated pro-inflammatory cytokines [32,44]. However, it is not known how sex differences in immune (cytokines and

CRP), stress hormone (cortisol) and neuroplasticity markers may be related to sex differences in AD or AD biomarkers. Biomarkers are highly sought after to predict disease onset and progression and to understand the possible underlying mechanisms of AD to develop better treatments. Therefore, we investigated potential physiological biomarkers (immune, stress, neuroplasticity) that may explain sex differences in AD and in people at risk for AD, possessing the APOEε4 allele, using the ADNI database.

Aging biomarkers also include epigenetic alterations, and are associated with a variety of pathologies and adverse health conditions, including normal cognitive aging and neurodegenerative phenotypes such as AD [45–48]. Recently, molecular biomarkers of aging known as "epigenetic clocks" have been developed based on DNA methylation signatures [45,46]. Epigenetic age or "DNAmAge" is a measure of the biological age of a sample (cell or tissue), and can be calculated across a range of tissues and time points, providing an accurate estimation of a sample's chronological age based on the presence or absence of methylation at the 5' carbon of informative CpG dinucleotides throughout the human genome [46]. Positive deviations of epigenetic age from chronological age (positive epigenetic age acceleration) reflect more rapid biological aging and have been associated with numerous factors including smoking, obesity, Parkinson's disease, Trisomy 21, and cancer [46,49–51], while negative deviations of epigenetic age from chronological age (negative epigenetic age acceleration) have been associated with high life-expectancy populations and memory retention [52,53]. In AD, epigenetic age acceleration of the frontal cortex was associated with amyloid load, neuritic plates, and cognitive decline [47]. Intra-individual DNA methylation profiles in peripheral tissue are correlated with the epigenetic signature in the brain, likely due both to identical genetic background affecting DNAme, and common signatures of epigenetic aging [54], thus it is

reasonable to hypothesize that epigenetic age acceleration may also be detectable in peripheral tissues such as blood in AD participants. In healthy individuals, aging males exhibit more positive epigenetic age acceleration than females in blood and buccal tissue, and multiple brain regions [55]; in AD and other diseases with a sex difference, it is possible that the underlying sex-specific pathological mechanisms may be reflected in epigenetic age acceleration measures – for example, in AD females could potentially have more positive epigenetic age acceleration than males.

Our aims were to first examine sex differences in cognitive ability, volume of the hippocampus (using different correction factors), neuropathological markers of AD (amyloid beta and tau), potential underlying physiological mechanisms (neuroplasticity, immune, stress) and epigenetic age in peripheral tissue, and how these may be affected by APOE genotype (number of ε4 alleles), and secondly by dementia status (cognitively healthy (CN), MCI, AD).

METHODS

ADNI database

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). For up-to-date information, see www.adni-info.org. Data used in this article were downloaded on or before Jan 16, 2019.

Statistical Methods: Sex and APOE genotype and sex and diagnosis

We included all participants who had the following baseline data: diagnosis in the ADNI database, cerebrospinal fluid (CSF) levels for amyloid beta, tau and phosphorylated tau (ptau), underwent a battery of neuropsychological tests, and had a brain MRI scan (total n = 1,460, n= 630 females, n=830 males; Table 1). Data included in our analyses were: demographics (age, years of education, and ethnicity), baseline diagnosis (cognitively normal, CN; early MCI, EMCI; late MCI, LMCI; or AD), number of APOE ε4 alleles (0, 1 or 2), ADNI executive function z-scores, ADNI memory z-scores [using data from the ADNI neuropsychological battery and validated in 56,57], hippocampal volume (mm^3), CSF amyloid beta (pg/ml), CSF tau (pg/ml), and CSF p-tau (pg/ml). The executive function score included WAIS-R Digit Symbol Substitution, Digit Span Backwards, Trails A and B, Category Fluency, and Clock Drawing [57]. The composite memory score included Rey Auditory Verbal Learning Test, AD Assessment Schedule - Cognition, Mini-Mental State Examination, and Logical Memory data [56]. A small subset of participants also had inflammatory markers measured in CSF (N = 279) and plasma (N = 527) listed in Table 2A. Plasma and CSF samples from the ADNI study were collected at baseline in the morning after an overnight fast. Processing, aliquoting and storage were performed according to the ADNI Biomarker Core Laboratory Standard Operating Procedures. Inflammatory markers were measured using a commercially available multiplex proteomic panel (Human Discovery Multi-Analyte Profile; Luminex xMAP) developed by Rules-Based Medicine (Austin, TX), that measures a variety of markers including cytokines, metabolic markers, and growth factors. The protocols used to quantify plasma and CSF analytes are described in Craig-Schapiro et al. [58] and Hu et al. [59]. We used the ADNI quality-controlled data for plasma and CSF provided by the ADNI Consortium. Sex differences in hippocampal volume are influenced

by controlling factors such as intracranial volume [60,61]. In the present study we corrected hippocampus volume using two different methods. The first method used the method in Mormino et al. [62] and Jack et al. [63] using the residuals of the linear regression between hippocampal volume and total intracranial volume. We also compared another widely used volume correction method [27,meta analysis by 61 and references herein,64] by dividing hippocampal volume with intracranial volume (the ratio method) to compare the different correction methods.

We compared all available data for each study variable between the sexes using the Wilcoxon rank sum test for continuous variables and Fisher's exact test for categorical variables. We used general linear models to determine the relationships between (1) sex and APOE genotype or (2) sex and dementia diagnosis as predictor variables, and cognitive ability, corrected hippocampal volume, and biomarkers as dependent variables. Due to the sample size, we were not able to study sex, APOE and diagnosis in one model. All models included age as a covariate. All models initially included an interaction between sex and APOE genotype or sex and dementia diagnosis; if this interaction was not significant, it was removed from the model to estimate the main effects of sex and APOE genotype or diagnosis. Significance was based on the likelihood ratio test, and all p-values for comparisons of sex and either APOE or diagnosis for all outcomes combined were corrected for multiple testing using the Benjamini-Hochberg false discovery rate method with the family-wise error rate set to 0.05 [65]. In total, three p-values per dependent variable were included in each set of models (interaction term and main effects of sex and APOE or diagnosis) resulting in 78 p-values corrected (26 dependent variables; Tables S1 to S4) for each of the models (sex and APOE and sex and diagnosis). All regression analyses were carried out in R v3.5.1 [66].

Statistical Methods: Epigenetic Age

We used DNAme data quantified with the Illumina Infinium HumanMethylationEPIC BeadChip array ("EPIC" array) for 1905 blood samples from 640 unique ADNI participants (n=284 females, n= 356 males) [67] with CN, MCI and AD diagnosis. DNAme IDAT files were read into R v3.5.1 [66] using the 'minfi' package, and annotated with the most recent version of the EPIC manifest, the Infinium MethylationEPIC v1.0 B4 Manifest File, (available from https://support.illumina.com/downloads.html) [68,69]. We excluded 11 low quality samples from 9 unique participants from further analyses on the basis of having a median methylated or unmethylated probe intensity <10.5 [68,69], the remaining samples were background normalized and dye-bias adjusted with normal exponential out-of-band ("noob") normalization [70]. DNAme data were converted to beta values and the biological sex of all samples was confirmed by clustering samples on all array probes mapping to the X and Y chromosomes. Beta values were calibrated to Horvath's 21,368-probe training dataset, and epigenetic age was calculated using R code modified for compatibility with the EPIC array using the 334/353 epigenetic clock probes present on the array from https://horvath.genetics.ucla.edu/ [46,71]. The missing DNAme values at these CpG sites can also be imputed based on the k-nearest neighbors method. We observed a high correlation between epigenetic age values calculated with the missing probes removed versus imputed with k=10 (R=0.99, p<2.2e-16), in agreement with previous reports [72,73]; we therefore chose to remove missing probes.

Prior to statistical analyses we removed all technical replicate samples. Epigenetic age acceleration was calculated as the residual of epigenetic age regressed on chronological age and technical batch covariates: the laboratory collection site at which blood samples were drawn, and

EPIC microarray chip and row. Intrinsic epigenetic age acceleration, a measure designed to be independent of age-related changes in whole blood cell-type proportions, was calculated as described in Chen et al. [74] as the residual of epigenetic age regressed on chronological age, technical covariates of collection site, row, and chip, and the proportions of six blood cell types (CD8T, CD4T, NK, B cells, monocytes, and granulocytes) estimated from noob-normalized DNA methylation data with the Houseman algorithm [75]. ADNI is a longitudinal study, and participants contributed between 1-3 blood DNAme samples within the 2-year collection period. Because these data collected over two years fell within the median 3.6-year error of Horvath's epigenetic clock, it therefore could not be meaningfully evaluated longitudinally. As such, for all epigenetic age measures reported and analyzed herein, we have calculated the average epigenetic age acceleration measure per participant from all available time points and performed all statistical analyses on these mean values.

Statistical analyses of epigenetic age acceleration were conducted using data from the remaining 640 participants (see Table 2B). To determine if epigenetic age acceleration or intrinsic epigenetic age acceleration differed by sex or dementia diagnosis, we used unbalanced two-way ANOVA designs. APOEε4 genotype and CSF biomarker (amyloid beta, tau and p-tau) data was available from the ADNI repository for a smaller subset of participants with matched EPIC DNAme data, (n=533, see Table 2C). Due to the small size of this cohort, we collapsed APOE genotype into two groups: (1) samples carrying any ε4 alleles (homozygous ε4/ε4 and heterozygous ε4/-) and (2) samples with no ε4 risk alleles (-/-). We subsequently used linear regression to test whether presence of an APOEε4 allele, tau, dementia diagnosis, or sex were significantly associated with epigenetic age acceleration. Amyloid beta and p-tau were not

included in this model due to collinearity between amyloid beta, tau, and p-tau CSF concentrations (see Supplementary Table S5).

RESULTS

Demographic and biomarker information

Table 1 gives a summary of the variables for the overall data set (N=1460). Overall, females were significantly younger and had fewer years of education than males (P<0.0001 for both). There were more white males than white females in our sample and there were more non-white females compared to non-white males (P<0.05). In terms of APOE genotype, there were no sex differences in distribution of APOE genotype with 11% females and 12% of males possessing two alleles of APOEε4. In the overall data set, the proportion of participants in each of the diagnosis categories was significantly different for females and males (P<0.05). More females were cognitively normal than males (26.7% compared to 20.8%, unadjusted P = 0.01) and although there were more females with a baseline diagnosis of AD compared to males (23.7% compared to 21.7%, unadjusted P = 0.41), this was not significant. However, there were more males with a diagnosis of late MCI (39.5% versus 32.5%, unadjusted P=0.007) and early MCI (18.0% versus 17.1%, unadjusted P=0.74) compared to females, although not significantly.

Because not all data were available for each subject we created a summary table for the participants: with CSF biomarkers (Table 2A; N=279), with whole blood EPIC DNAme data (Table 2B; N=640) and with matched EPIC data and measured CSF biomarkers (Table 2C; N=533). Among those with measured CSF biomarkers, demographics were very similar as per results from overall data set in Table 1 (see also legend of Table 2). For the data applicable to the participants with available EPIC DNAme data (Table 2B) and participants with EPIC DNAme

data and CSF biomarkers (Table 2C), most of the demographics were similar to the entire data set except the proportion of participants in each of the diagnosis categories was not significantly different between females and males.

In the overall data set (Table 1), females had a smaller uncorrected (P<0.0001) and corrected hippocampal volume using the regression method in [62] (P<0.0001) but larger corrected hippocampal volume using the ratio method with intracranial volume (P<0.0001) [27,61,64]. Females also had greater CSF amyloid beta (P=0.016), tau and p-tau (P=0.002 and 0.007 respectively), and higher memory function z-scores than males (P=0.0006; Table 1). Biomarkers in the CSF were measured in a subset of participants (Table 2A). In this smaller cohort, females and males had similar levels of CSF CRP, CD 40 antigen and IL-6 receptor. However, females had lower CSF cortisol (P=0.008), interleukin-3 (P=0.001), interleukin 8 (P=0.001), interleukin-16 (P=0.004), immunoglobulin A (P<0.0001), and intercellular adhesion molecule (P=0.0001) compared to males (Table 2A).

Sex and APOE genotype are associated with changes in memory, hippocampus volume, AD and CSF inflammatory markers

Our first aim was to investigate whether sex and APOE genotype interact to influence cognitive ability, volume of the hippocampus, and biomarkers of AD and inflammation. There were significant interactions between sex and APOEε4 genotype for CSF tau, p-tau, and IL-16 (P=0.001, 0.002 and 0.03 respectively; Table 3). Tau and p-tau levels were significantly higher in females with one or two alleles of APOEε4 compared to males (Fig 1 A and B). Although CSF p-tau and tau levels also increase in males with APOEε4 genotype, they do not rise to the same extent as in females. CSF IL-16 levels were significantly lower in females with no

APOEε4 alleles compared to males, whereas levels were similar between the sexes with one or two APOEε4 alleles (Fig 1 C).

Both sex and APOE genotype were independently (main effects of sex or APOE genotype) associated with memory z-scores and corrected hippocampal volume (Table 3). Females had higher memory z-scores across all APOE genotypes (P<0.0001; Fig 1 D). Curiously, females had smaller or larger hippocampi than males depending on the correction method used: females had smaller corrected hippocampal volume based on a regression method [62] (P<0.0001; Fig 1 E) but larger hippocampal/intracranial volume compared to males (P<0.0001; Supplementary Table S1). These data suggest it is important to consider different correction factors when studying sex differences in volumes of brain structures. Lower memory z-scores were associated with increasing number of APOEε4 alleles in both sexes (P<0.0001). Similarly, corrected hippocampus volume (independent of method of correction) was significantly lower with increasing number of APOEε4 alleles in both sexes (Ps<0.0001). Increasing APOEε4 alleles was also associated with lower executive function z-scores (P<0.0001), lower CSF amyloid beta (P<0.0001), and lower CSF CRP levels (P=0.009) (Table 3; Fig 1 F-H), however there was no additional association of these variables with sex. Finally, results were similar for biomarkers in plasma (Supplementary Table S3) with the exception of a significant relationship between plasma CRP and sex. Males had lower plasma levels of CRP compared to females (adjusted P=0.003).

Sex and diagnosis are associated with changes in memory, hippocampus volume, AD and CSF inflammatory markers

We next tested whether sex and dementia status (CN, MCI, and AD) influenced cognitive ability, corrected hippocampal volume, and CSF biomarkers of AD and inflammation. There were no significant interactions between sex and diagnosis for any of the tested variables (memory, executive function, corrected hippocampal volume, CSF tau, p-tau, amyloid beta, and CSF and plasma inflammatory markers). However, overall both sex and diagnosis were independently associated with memory z-scores, corrected hippocampal volume and CSF tau and p-tau (Table 4). Females had higher memory z-scores ($P<0.0001$), smaller corrected hippocampus volume ($P<0.0001$) but larger hippocampus/intracranial volume ratio ($P<0.0001$; Supplementary Table S2), and higher tau and p-tau compared to males ($Ps<0.0001$), irrespective of diagnosis (Table 4). As expected, increasing severity of diagnosis was associated with lower memory and executive function z-scores ($Ps<0.0001$), smaller corrected hippocampus volume ($Ps<0.0001$; independent of correction method), lower CSF amyloid beta ($P<0.0001$), and higher CSF tau and p-tau irrespective of sex ($Ps<0.0001$; Fig 2 A-F).

We found that although females had lower CSF levels of interleukin 8 (IL-8; $P=0.03$), immunoglobulin A (IgA; $P<0.0001$), interleukin 16 (IL-16; $P=0.07$), and intercellular adhesion molecule 1 (ICAM1; $P=0.008$), controlling for age, compared to males, there was no association between these variables and diagnosis (Table 4; Fig 2 G-J). The results for biomarkers and inflammatory markers were different in plasma (Supplementary Table S4) with females having higher levels of plasma ICAM1 compared to males ($P=0.05$) and no sex differences in plasma IL-8, IgA and IL-6 levels. We found a significant relationship between plasma CRP and sex (adjusted $P=0.01$), immunoglobulin E (IgE) and sex ($P<0.0001$), IL-18 and sex ($P=0.001$), and

also between plasma cortisol and baseline diagnosis (adjusted P=0.005; Fig 2 K and L). Males had lower levels of plasma CRP compared to females and late MCI and AD resulted in lower plasma CRP levels compared to CN (adjusted P=0.04). Plasma cortisol was lower in late MCI compared to CN but higher in AD compared to CN. In summary, although we detected associations between sex and diagnosis and various parameters, we did not find evidence for a clear sex and diagnosis interaction.

Epigenetic age, sex, dementia diagnosis, and AD biomarkers

We investigated the hypothesis that sex and dementia diagnosis affect epigenetic age acceleration in blood samples of ADNI participants (see Table 5). Epigenetic age acceleration was not associated with sex, dementia diagnosis (CN, EMCI, LMCI, and AD), or the interaction of sex and diagnosis after multiple test correction (Fig 3). Intrinsic epigenetic age acceleration was also not significantly associated with participant sex, diagnosis, or their interaction term.

To assess the effect of sex and more broadly defined dementia-associated cognitive impairment on epigenetic age acceleration, we compared epigenetic age acceleration between participants with any form of clinically ascertained cognitive impairment (AD + LMCI + EMCI, n=423, proportion female 41%) and those without (CN, n=217, proportion female 50%). By two-way unbalanced ANOVA neither sex, dementia status, nor their interaction were significantly associated with epigenetic age acceleration after correction for multiple comparisons.

Matched biochemical data including APOEε4 genotype and CSF concentrations of amyloid beta, tau, and p-tau was available for a subset of participants with EPIC DNAme data (n=533). Based on the hypothesis that epigenetic age acceleration may be more strongly associated with concentrations of pathologically relevant compounds than with diagnosis, we

assessed the impact of sex, APOEε4 genotype, CSF amyloid beta concentration, CSF tau and p-tau concentration on epigenetic age acceleration and intrinsic epigenetic age acceleration with linear regression. None of these variables were significantly associated with epigenetic age acceleration (Table 6; results for intrinsic epigenetic age acceleration not shown).

In addition to dementia diagnosis for all participants, we also had access to two composite scores designed by ADNI collaborators to reflect executive function and memory; these z-scores have been demonstrated to be independently predictive of the transition from MCI to a formal diagnosis of AD [56,57]. By a two-way unbalanced ANOVA models investigating the effect of sex and memory z-scores on epigenetic age acceleration, neither sex (P=0.248), memory score (P=0.486), nor their interaction (P=0.227) were associated with epigenetic age acceleration. In a similar model, neither sex (P=0.260), executive function (P=0.105), or the interaction term of sex and executive function (P=0.153) were associated with epigenetic age acceleration.

DISCUSSION

In the present study, we found that tau-related pathology in the CSF was disproportionately elevated by APOEε4 genotype in females compared to males. However, diagnosis and APOE genotype were independently associated with reduced memory scores, corrected hippocampal volume and reduced CSF amyloid beta which was similar in males and females. Furthermore, there were main effects of sex as females had lower CSF and plasma cytokines (IL-8, IL-16, IL-18) and CSF and plasma immunoglobulins (IgA, IgE, respectively) but higher plasma CRP and tau-related pathology compared to males, regardless of diagnosis and APOE genotype. Interestingly, depending on the correction method, females had either smaller

or larger hippocampus volume compared to males. Females had better memory scores which may contribute to their delayed diagnosis [76]. Finally, we found no differences in epigenetic age acceleration by dementia diagnosis or sex in this cohort of samples with available whole blood EPIC DNAme data. In this ADNI cohort, significantly more males presented with a diagnosis of MCI while there was no sex difference in the number of participants diagnosed with AD, suggestive of sex differences in transitions to MCI and to AD. It is interesting to note that population-based studies find males have more MCI whereas females have more AD [77–79], although not all studies agree in the higher prevalence or incidence of AD in females [reviewed in 4,80]. Previous work has demonstrated sex differences in rates of AD and symptoms of AD [4,77,reviewed in 81], and the current study also suggests that biomarkers of AD may be different between males and females between genotypes, and this should be considered in future studies and researchers should be cautioned to use sex as a biological variable in all analyses.

Females show greater tau neuropathology disproportionately affected by APOE genotype

In the present study, we found that females have significantly higher baseline tau and p-tau levels in CSF than males and these are indicative of the formation of neurofibrillary tangles and AD pathology [82,83]. This is in agreement with a recent ADNI study [64, but see an earlier ADNI study 9] and with animal models [84]. Intriguingly, we also found that levels of tau and p-tau were disproportionately elevated with APOEε4 allele expression in females compared to males and this is in agreement with a previous ADNI study that included cognitively healthy individuals only [22]. A meta-analysis that included data from ADNI also found a stronger association between APOEε4 and tau levels in females compared to males [85] but interestingly this association is not present in all ADNI studies which may depend on sample size, type of

statistical analysis, and the diagnosis groups included in the analyses [23,24]. Previous studies indicate that females with the APOEε4 allele are at a greater risk for developing AD than are males with this allele [86], and sex differences in tau and p-tau may be one underlying mechanism by which this occurs but further replication with additional populations is needed. In females (65-75 years of age) one allele of ε4 increases the risk of AD by 4-fold relative to males, indicating that genotype may affect females differently [21]. Levels of CSF tau are hypothesized to increase after CSF amyloid beta declines and amyloid beta aggregates and deposits in the brain [82]. However, in this study although we found sex differences in CSF tau and p-tau levels, no significant differences were seen in CSF amyloid beta after controlling for age (see below) indicating that the pathway may be different in females compared to males or that the timeline of tau and amyloid beta deposition may not be consistent.

Females are more likely to be diagnosed with AD in Europe and Asia, although this sex difference may depend in part on geographic location as the sex difference is not always observed in studies from the United States [4,77,reviewed by 81]. Nevertheless, the disproportionate effect of APOEε4 genotype on tau-related pathology in females supports the idea that females have a higher burden of the disease dependent on APOE genotype. On the other hand, males are more likely to be diagnosed with MCI compared to females [18,77,80], while females progress faster from MCI to AD [13]. Sex differences in tau-related pathology found in the current study may be the underlying mechanism for this accelerated transition.

Sex differences in hippocampal volume depend on correction method. Females have better memory scores than males that may have been driven by verbal memory

In the present study, we found that increasing APOEε4 alleles and AD diagnosis was associated with reduced corrected hippocampal volume, memory and executive function scores consistent with past literature [15,16,18,87–92]. Surprisingly, although females have higher levels of tau and p-tau, they presented with better memory and executive function scores than males, regardless of diagnosis and APOE genotype. Depending on the correction method, females had larger or smaller hippocampal volume compared to males. Females had smaller corrected hippocampal volume using the residuals of the linear regression between hippocampal volume and total intracranial volume [method by 62,63] but larger ratio of hippocampal/intracranial volume compared to males [method used in 27,61,64]. These results have important implications for future studies trying to understand sex differences in AD (and other neurodegenerative diseases) as they lead to very different conclusions regarding hippocampal plasticity. We suggest future research should report uncorrected as well as corrected volumes using multiple methods. Previous studies have suggested that there are sex differences in hippocampal volume, favoring males, but the sex differences depend on whether hippocampal volume is corrected for by intracranial volume or total brain volume and the method of correction [61]. In a number of studies, including the present study, males have a larger hippocampus without correcting for intracranial volume [27,61,64,93–96]. However after correcting for intracranial volume using the regression model, studies have found females have larger [94], smaller (present study) or no difference [61,93] in corrected hippocampal volume compared to males. Using the ratio method, Sohn et al. [27], Sundermann et al. [64], Murphy et al. [95] and the current study found that females had larger hippocampal volumes compared to males. In their meta-analysis, Tan et al. [61] conclude that sex differences in hippocampal volume are modest and highly depend on correction method and this is supported by our

findings. Regardless of hippocampal volume, volume loss is greater in aging females [7,26,95] and in females with one or two APOEε4 alleles [97]. Although in the present study we did not examine longitudinal data, we found that increasing APOEε4 alleles reduced corrected hippocampal volume similarly in males and females. In contrast, when CN, MCI and AD individuals were analysed separately in the ADNI database, APOEε4 was associated with a smaller corrected hippocampal volume in CN males only, controlling for age and education [64]. In addition, also using the ADNI database, Koran et al. [26] found that females with low CSF amyloid beta had more hippocampal atrophy and faster decline in memory and executive function than males and this sex difference was more pronounced in APOEε4 carriers. Therefore, sex and APOE genotype can interact to affect corrected hippocampal volume reduction with age in certain subgroups and across time (e.g., in CN or individuals with low CSF amyloid beta). Differences in results between studies are likely due to differences in statistical analyses (e.g., analysing diagnosis groups separately, partitioning the data based on amyloid beta levels, and differences in covariates included) and/or whether longitudinal data analyses are included.

We found females had better composite memory scores (but not executive function scores) than males, regardless of diagnosis and APOE genotype. Previous studies have found that females have better verbal memory in cognitively normal individuals [94], and in MCI and AD ADNI cohorts compared to males [64,98]. Here we used the ADNI memory score developed by Crane et al. [56] to detect abnormal memory including language, attention, and logical memory so it is possible that verbal memory may be driving the sex difference favouring females in the present study. In contrast, Buckley et al.[99] found no sex differences using a composite cognitive score that includes memory and executive function (Preclinical Alzheimer's Cognitive Composite score with semantic processing, PACC5) using ADNI and two other cohorts. In this

study using the current ADNI cohort, males were slightly more educated than females, and although we did not use education as a covariate, one would expect education levels would have positive effects on memory, suggesting that education is not a factor for the observed sex difference in memory. Altogether, we found that in females tau pathology was increased but memory scores, which included verbal memory, were higher compared to males suggesting females have a reserve against brain damage that delays either the onset of cognitive decline [100] or diagnosis [76]. However, once cognitive decline begins, females show higher rates of declines compared to males [9,10,99] perhaps because the underlying pathology is elevated in females.

AD affects amyloid beta similarly in both sexes

We found that AD diagnosis was associated with lower CSF amyloid beta, as expected, and this was irrespective of sex, which indicates greater amyloid deposition with AD [83]. These findings are consistent with data from studies in cognitively healthy individuals [94,99]. Other studies have found using PET that males have higher amyloid beta levels or lower amyloid beta burden compared to females dependent on APOE genotype [64] or in cognitively normal adults in the anterior cingulate [93]. In this study, we used CSF amyloid beta data which detect abnormal amyloid deposition earlier than amyloid beta by PET [reviewed in 82]. Thus, taken together, sex differences in amyloid beta may be detected in specific brain regions and later in the disease, although more research is needed investigating sex differences in AD biomarkers.

Females have higher CRP levels but lower cytokine and immunoglobulin levels compared to males

In this study, we investigated whether sex interacted with APOE genotype or dementia diagnosis to influence inflammatory, neurotrophic and neuroplasticity markers. We found that plasma CRP, a widely used inflammatory and cardiovascular marker [101,102], was affected by sex and APOE genotype. Females, regardless of diagnosis or APOE genotype, had significantly higher plasma CRP relative to males, consistent with findings in healthy individuals [35]. Higher levels of peripheral CRP may suggest higher systemic inflammation in females, which is associated with an increased risk in all-cause dementia [103]. In contrast, APOEε4 genotype and diagnosis decreased circulating CRP levels. The effect of APOEε4 genotype is consistent with previous research in large population studies [104,105]. Recent meta-analyses, without regard to sex, did not find differences in peripheral levels of CRP in AD compared to control patients [106,107]. However, in patients with mild and moderate dementia only, CRP levels were lower compared to the healthy control group [106]. In healthy individuals, higher levels of plasma CRP in midlife are associated with a higher amyloid burden later in life in males but not females [36]. However, despite this finding, higher systemic inflammation (including CRP) was associated with greater cognitive decline later in life in both sexes in healthy individuals [37]. It is possible that sex differences in inflammation earlier in life have long term effects on the transition to MCI and/or AD. To our knowledge, no other study has examined sex differences in CRP in relation to AD.

We also found that CSF IL-16 was affected by sex and APOE genotype. CSF IL-16 levels were lower in females with no APOEε4 alleles compared to males, but with increasing number of ε4 alleles, no sex differences were detected. IL-16 has been implicated in AD [108] and IL-16 levels decrease with disease severity [analysis without regard to sex; ,109]. In this ADNI cohort, IL-16 levels were not affected by diagnosis but our results suggest that APOE

genotype can modulate levels in a sex-dependent way. We also found biomarkers that were affected by sex but not diagnosis or APOE genotype for example, females had lower CSF levels of ICAM1 compared to males, but there was no influence of APOE genotype or diagnosis. Consistent with our findings, ICAM1 serum levels were lower in healthy females compared to males [110]. ICAM1 is a type of adhesion molecule associated with microvascular endothelial activation [111] and plasma ICAM1 levels [but not CSF levels; ,112] were higher in patients with AD [112–114]. However, it is intriguing that females have lower CSF levels of cytokines (IL-8, IL-16, IL-18), and immunoglobulins (IgE and IgA) but higher tau pathology compared to males. Neuroinflammation is associated with AD but it can have both beneficial and detrimental roles [115]. Increased expression of pro-inflammatory cytokines contributes to neuronal loss, while anti-inflammatory effects contribute to amyloid beta clearance [116]. In AD mouse models, some pro-inflammatory mechanisms reduced plaque pathology, while anti-inflammatory cytokines increased amyloid beta deposition [117–122]. It has been suggested that there are beneficial pro-inflammatory mechanisms and detrimental anti-inflammatory mechanisms in AD [116]. It is possible that males and females have varying levels of beneficial vs detrimental immune responses which can affect how the disease progresses in each of the sexes but it is also important to remember that CSF levels may not match levels in different regions of the brain.

Sex, AD and biochemical markers do not affect blood epigenetic age acceleration

We did not observe an association between either sex or diagnosis and epigenetic age acceleration or intrinsic epigenetic age acceleration. To our knowledge, no other study has similarly probed epigenetic age acceleration in peripheral tissue in the presence of AD, or whether epigenetic age acceleration in AD is associated with sex.

This study was partially undertaken to investigate whether epigenetic age acceleration that has been associated with the AD brain is reflected in peripheral tissues. Levine et al. [47] have previously demonstrated increased epigenetic age acceleration in AD, however Levine's study was conducted on post-mortem prefrontal cortex tissue, and did not explicitly investigate the role of sex in epigenetic age acceleration [47]. While brain-blood methylation profiles are reasonably correlated (r=0.86) [54], DNA methylation profiles of peripheral tissues are imperfect representatives of the brain, and do not recapitulate all epigenetic alterations with high fidelity. Thus, our findings do not contradict the finding of increased epigenetic age acceleration in the presence of AD in the prefrontal cortex, but suggest that accelerated epigenetic aging in AD is not a pan-tissue phenomenon. Our finding of a lack of significant association between AD, biological sex, and epigenetic age acceleration in whole blood DNA methylation profiles could suggest a tissue-specific dysregulation of an epigenetic maintenance system, in which the brain epigenome is most strongly affected by AD [47]. The phenotype of patients affected by AD and global gene expression patterns of the APOE protein, with high expression in brain, and low expression in whole blood [123] further support this hypothesis.

Intriguingly, epigenetic age was observed to be lower on average than chronological age. Horvath's epigenetic clock was trained on DNAme data from older versions of the Illumina DNAme arrays with more limited genomic coverage; 19 of the CpG probes required to calculate epigenetic age via this method do not exist on the EPIC array. Two previous studies investigated the application of Horvath's epigenetic clock to EPIC data with conflicting results [73,124], the largest issue being chronic underestimation of epigenetic age due to the positive linear regression coefficients associated with the missing probes [124]. Both imputing and removing the missing probes from the array resulted in a chronic underprediction of epigenetic age with Horvath's

clock, suggesting that this is likely an artefact of the array platform and probe-set rather than the method chosen to deal with missing values, although it is possible that an adjustment factor could be devised to more accurately apply Horvath's clock to EPIC data. In future explorations of epigenetic age with EPIC DNAme array data this should be considered, as there are other epigenetic age predictors available that have been trained on EPIC data such as the PhenoAge and GrimAge clocks, although these tools have limitations as well; for example, both PhenoAge and GrimAge were trained only on blood DNAme data, as compared to the original pan-tissue epigenetic clock, and therefore may have limited applicability and relevance in other tissues [125,126].

Limitations

The ADNI cohort is not ethnically or socioeconomically diverse, being mostly composed of white (only 12 individuals were not-white) and highly educated individuals (average 15.69 years of education). As incidence, prevalence, and age of onset of AD varies by ethnicity [127–129] and education [130], our conclusions may not apply to more ethnically and socially diverse populations. In addition to sex, it is possible the underlying mechanisms of AD are different depending on ethnicity. APOEε4 genotype is a well-known AD risk factor and in this cohort the proportion of participants in the different APOEε4 allele groups is correlated with diagnosis. Future research with a larger cohort is required to test how sex, APOE genotype and diagnosis interact together in one model. Small sample size is also a limitation of the epigenetic analyses presented. Even in the larger 640-participant cohort, only 37 participants (5.78%) had an AD diagnosis, so statistical analyses were underpowered to detect subtle differences by diagnosis group. Additionally other pathologies in these participants, such as cancer, cardiovascular

disease, smoking status, or obesity may have influenced AD neuropathology, biomarkers and epigenomes and limited our interpretations.

CONCLUSION

As expected, more females presented with a diagnosis of AD whereas more males presented with MCI diagnosis compared to the opposite sex. AD biomarkers (CSF tau and p-tau but not amyloid beta) were disproportionately affected by APOE genotype in females compared to males supporting the idea that females share a higher burden of the disease. Interestingly, although females in this cohort had elevated AD biomarkers, they also had higher memory function scores compared to males, regardless of APOE genotype and dementia diagnosis. Therefore, it is possible that females may have a reserve that protects the brain from damage to delay cognitive decline or delay diagnosis. Finally, we found that females had lower cytokine and immunoglobulin levels but higher CRP levels compared to males. Together our work suggests that that the underlying physiology of aging and AD may be sex-specific.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

FIGURE CAPTIONS

Figure 1. A. CSF tau (pg/ml), B. CSF p-tau (pg/ml), C. CSF IL-16 (pg/ml), D. ADNI memory z-scores, E. corrected hippocampal volume (hippocampal volume/intracranial volume), F. ADNI executive function z-scores, G. CSF amyloid beta (pg/ml), and H. CSF C-reactive protein (CRP; µg/ml) in ADNI participants by sex and number of APOEε4 alleles (0, 1, 2 alelles).

Figure 2. A. ADNI memory z-scores, B. corrected hippocampal volume (hippocampal volume/ intracranial volume), C. CSF tau (pg/ml), D. CSF p-tau (pg/ml), E. CSF IL-16 (pg/ml), F. CSF IL-8 (pg/ml), G. CSF IgA (mg/ml), H. CSF Intercellular adhesion molecule (ICAM1; ng/ml), I. ADNI executive function z-scores, J. CSF amyloid beta (pg/ml), K. plasma C-reactive protein (CRP; µg/ml), and L. plasma cortisol (ng/ml) in ADNI participants by sex and diagnosis (CN, EMCI, LMCI, AD). CN, cognitively normal; EMCI, early mild cognitive impairment; LMCI, late mild cognitive impairment; AD, Alzheimer's disease.

Figure 3. Universal epigenetic age acceleration does not differ statistically significantly by participant sex or diagnosis (CN, EMCI, LMCI, AD) in this ADNI cohort. CN, cognitively normal; EMCI, early mild cognitive impairment; LMCI, late mild cognitive impairment; AD, Alzheimer's disease.

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Figure 1

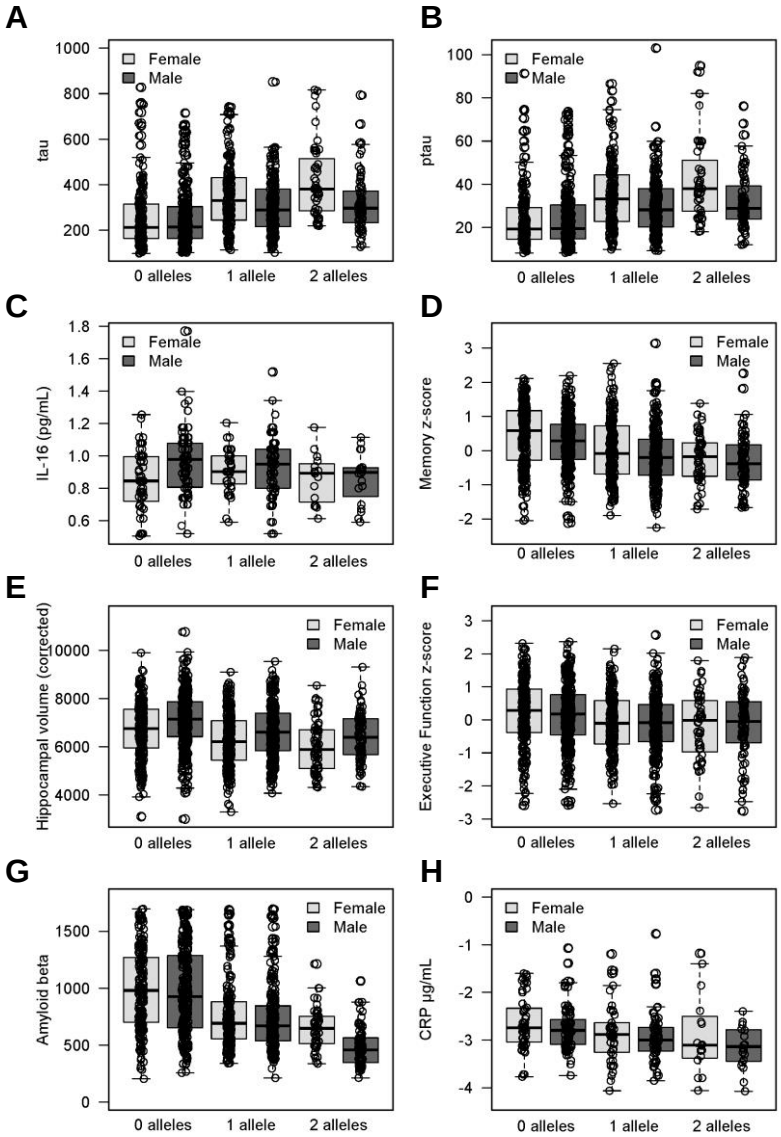
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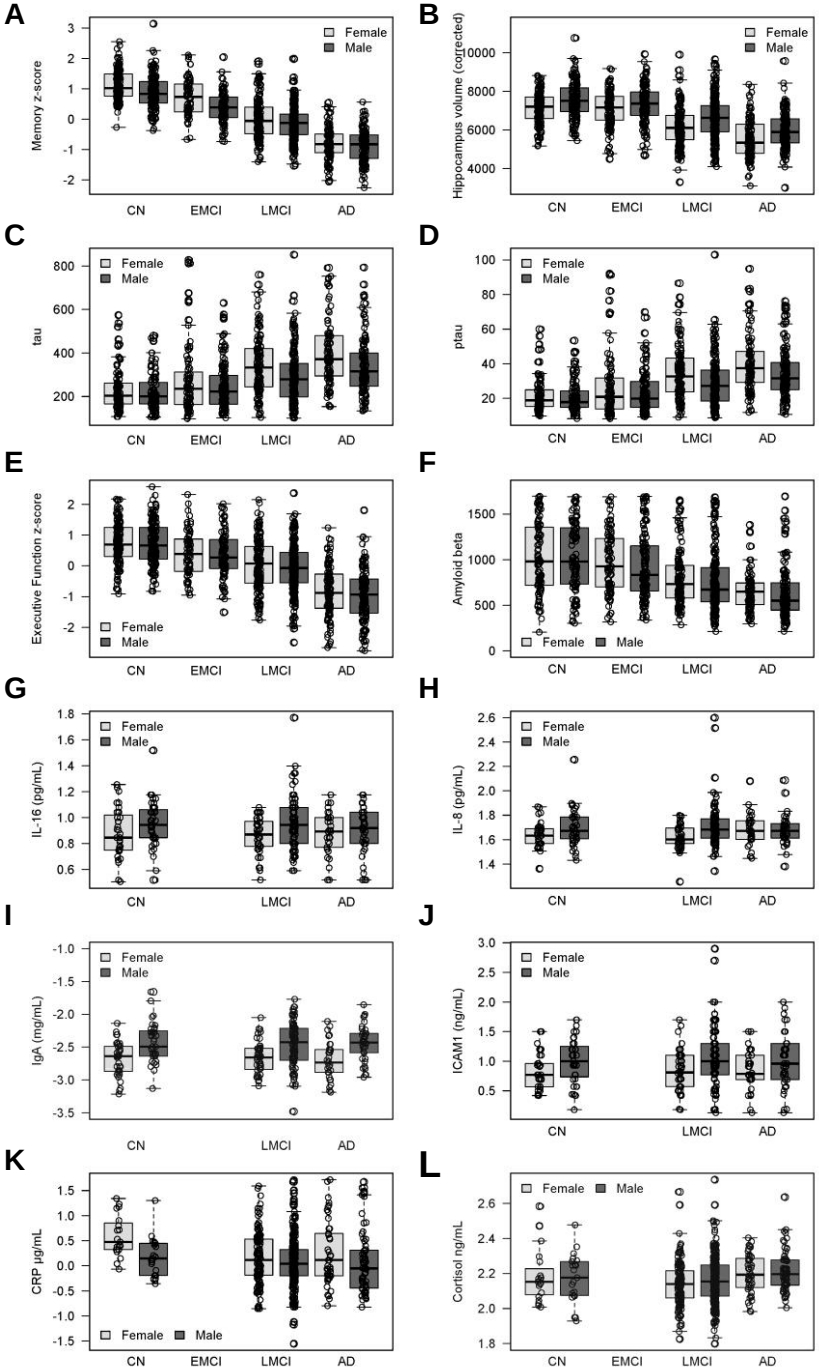
Figure 2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IL-16 (pg/mL)
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Cortisol ng/mL

Figure 3

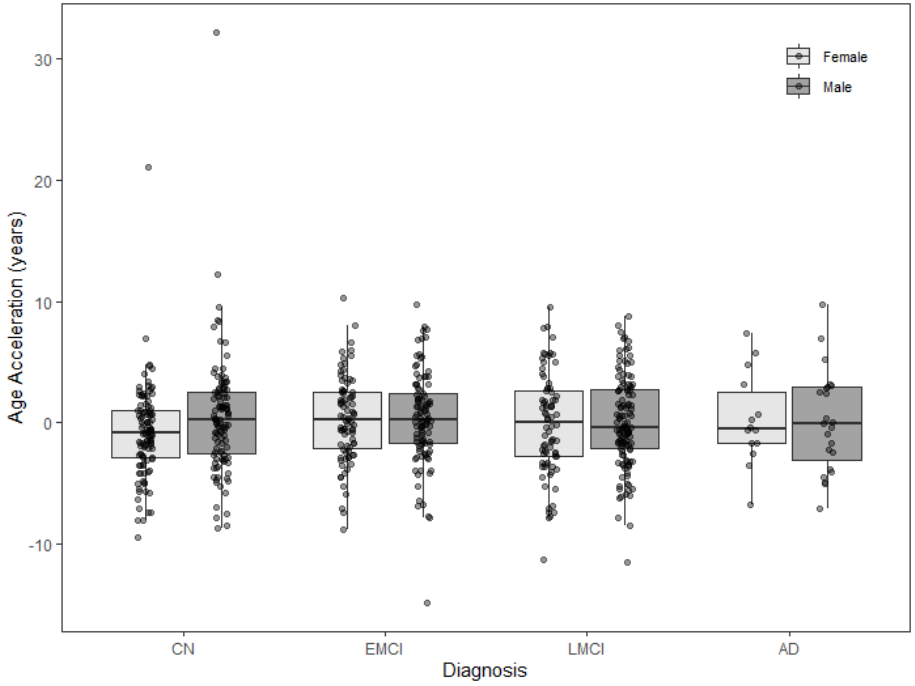
Female
Male
30
20
10
0
-10
Age Acceleration (years)
CN
EMCI
LMCI
AD
Diagnosis

Table 1. Demographic and clinical information for all participants and subdivided by sex. Biomarkers for AD are from cerebrospinal fluid. P-values adjusted for age are presented for easier comparison and are taken from the linear model of sex and APOE genotype (Table 3). Volume of the hippocampus was corrected using a regression method or using a ratio with intracranial volume (see methods for more details).

		Sex			
	Total	**Female**	**Male**	**P-value**	**P-value (adjusted for age)**
	No. 1,460	**No. 630**	**No. 830**		
Age					
Mean (SD)	74.13 (±7.25)	73.15 (±7.28)	74.87 (±7.14)	< 0.0001	
Education (years)					
Mean (SD)	15.83 (±2.88)	15.15 (±2.79)	16.34 (±2.85)	< 0.0001	
Ethnicity					
White	1,352 (92.60%)	573 (90.95%)	779 (93.86%)	0.043	
Not white	108 (7.40%)	57 (9.05%)	51 (6.14%)		
Baseline diagnosis					
CN	341 (23.4%)	168 (26.7%)	173 (20.8%)	0.013	
EMCI	257 (17.6%)	108 (17.1%)	149 (18.0%)		
LMCI	533 (36.5%)	205 (32.5%)	328 (39.5%)		
AD	329 (22.5%)	149 (23.7%)	180 (21.7%)		
APOEε4 allele number					
0	702 (48.08%)	300 (47.62%)	402 (48.43%)	0.8	
1	574 (39.32%)	252 (40.00%)	322 (38.80%)		
2	170 (11.64%)	70 (11.11%)	100 (12.05%)		
Missing	14 (0.96%)	8 (1.27%)	6 (0.72%)		
Volume of hippocampus (uncorrected, mm^3)					
Mean (SD)	6659.47 (±1176.42)	6446.71 (±1169.97)	6822.86 (±1155.87)	< 0.0001	
Missing	226 (15.48%)	94 (14.92%)	132 (15.90%)		
Volume of hippocampus (regression corrected)					
Mean (SD)	6659.47 (±1174.34)	6447.09 (±1167.90)	6822.57 (±1153.83)	**< 0.0001**	**<0.0001**
Missing	226 (15.48%)	94 (14.92%)	132 (15.90%)		
Volume of hippocampus/intracranial volume					
Mean (SD)	0.00436 (±0.00080)	0.00454 (±0.00082)	0.00423 (±0.00076)	**< 0.0001**	**<0.0001**
Missing	226 (15.48%)	94 (14.92%)	132 (15.90%)		
Amyloid Beta					
Mean (SD)	830.97 (±358.04)	856.41 (±346.87)	812.44 (±365.16)	**0.016**	0.38
Missing	513 (35.14%)	231 (36.67%)	282 (33.98%)		
Tau					
Mean (SD)	294.38 (±137.27)	314.56 (±152.70)	279.70 (±122.91)	**0.002**	**<0.0001**
Missing	513 (35.14%)	231 (36.67%)	282 (33.98%)		
PTau					
Mean (SD)	28.89 (±15.31)	30.87 (±16.95)	27.44 (±13.83)	**0.007**	**<0.0001**
Missing	513 (35.14%)	231 (36.67%)	282 (33.98%)		
Executive Function (ADNI_EF)					
Mean (SD)	0.02 (±0.96)	0.06 (±0.97)	-0.00 (±0.95)	0.20	**<0.0001**
Missing	311 (21.30%)	145 (23.02%)	166 (20.00%)		
Memory (ADNI_MEM)					
Mean (SD)	0.10 (±0.87)	0.21 (±0.94)	0.02 (±0.80)	**0.0006**	**<0.0001**
Missing	310 (21.23%)	145 (23.02%)	165 (19.88%)		

P-values are from Wilcoxon rank sum tests for continuous variables and Fisher's exact tests for categorical variables. Missing refers to number of individuals and the percent of the total cohort that had missing data for that variable

Table 2. Demographic and clinical information for subset of ADNI data subdivided by sex. A. Participants with measured biomarkers in cerebrospinal fluid (CSF), B. Participants with available whole blood Illumina HumanMethylationEPIC DNA methylation data, C. Participants with matched Illumina HumanMethylationEPIC DNA methylation array data and measured CSF biomarkers. In all three subdata sets, females were significantly younger and had fewer years of education than males. In data set A (but not B and C), more females (24.0 % compared to 21.8%) were diagnosed with AD, more females were cognitively normal (26.5% compared to 22.9%) and fewer females were diagnosed with late MCI compared to males (49.5% compared to 55.3%). In data set A, females had lower CSF cortisol, interleukin-3, interleukin 8, interleukin-16, immunoglobulin A, and intercellular adhesion molecule compared to males. Empty cells indicate data not available.

	A				**B**				**C**			
		Sex				**Sex**				**Sex**		
	Total No. 279	**Female No. 109**	**Male No. 170**	**P-value**	**Total No. 640**	**Female No. 284**	**Male No. 356**	**P-value**	**Total No. 533**	**Female No. 243**	**Male No. 290**	**P-value**
Age												
Mean (SD)	75.15 (±6.86)	73.75 (±6.69)	76.04 (±6.83)	0.007	75.63 (±7.68)	74.78 (±8.03)	76.31 (±7.32)	<0.0001	75.01 (±7.61)	74.31 (±8.10)	75.61 (±7.11)	0.0019
Education (years)												
Mean (SD)	15.69 (±2.95)	14.68 (±2.74)	16.34 (±2.90)	< 0.0001	16.21 (±2.70)	15.53 (±2.59)	16.75 (±2.68)	<0.0001	16.24 (±2.64)	15.57 (±2.49)	16.83 (±2.64)	< 0.0001
Ethnicity												
White	267 (95.70%)	103 (94.50%)	164 (96.47%)	0.55	627 (97.97%)	279 (98.23 %)	348 (97.75%)	0.78	521 (97.75 %)	238 (97.94 %)	283 (97.94 %)	0.99
Not White[ł]	12 (4.30%)	6 (5.50%)	6 (3.53%)		13 (2.03%)	5 (1.76%)	8 (2.25%)		12 (2.25 %)	5 (2.06 %)	7 (2.41 %)	
Baseline diagnosis												
CN	74 (26.5%)	35 (32.1%)	39 (22.9%)	0.051	217 (33.9%)	109 (38.38%)	108 (30.34%)	0.11	171 (32.08 %)	88 (36.21 %)	83 (28.62 %)	0.19
EMCI					186 (29.06%)	83 (29.23%)	103 (28.93%)		173 (32.46 %)	79 (32.51 %)	94 (32.41 %)	
LMCI	138 (49.5%)	44 (40.4%)	94 (55.3%)		200 (31.25%)	78 (27.46%)	122 (34.27%)		155 (29.08 %)	94 (38.68 %)	92 (31.72 %)	
AD	67 (24.0%)	30 (27.5%)	37 (21.8%)		37 (5.78%)	14 (4.23 %)	23 (6.46%)		34 (6.38 %)	13 (5.35 %)	21 (7.24 %)	
APOEε4 allele number												
0	134 (48.03%)	51 (46.79%)	83 (48.82%)	0.78	369 (57.66 %)	169 (59.51%)	200 (56.18%)	0.37	313 (58.72 %)	146 (60.08 %)	167 (57.59 %)	0.45
1	109 (39.07%)	42 (38.53%)	67 (39.41%)		220 (34.38%)	97 (34.15%)	123 (34.55%)		173 (32.46%)	80 (32.92 %)	93 (32.07 %)	
2	36 (12.90%)	16 (14.68%)	20 (11.76%)		51 (7.97%)	18 (6.34%)	33 (9.27%)		47 (8.82%)	17 (7.00%)	30 (10.34 %)	
Cortisol (ng/mL)												
Mean (SD)	16.05 (±6.04)	14.92 (±6.01)	16.78 (±5.96)	0.008								
C reactive protein (ug/mL)												
Mean (SD)	-2.83 (±0.56)	-2.77 (±0.64)	-2.87 (±0.51)	0.23								
CD40 antigen (ng/mL)												
Mean (SD)	-0.65 (±0.12)	-0.66 (±0.10)	-0.64 (±0.14)	0.12								
Interleukin 16 (pg/mL)												
Mean (SD)	0.91 (±0.18)	0.87 (±0.17)	0.94 (±0.19)	0.004								
Interleukin 3 (ng/mL)												
Mean (SD)	-2.22 (±0.32)	-2.28 (±0.29)	-2.17 (±0.34)	0.001								
Interleukin 6 receptor (ng/mL)												
Mean (SD)	-0.01 (±0.15)	-0.02 (±0.14)	-0.00 (±0.15)	0.30								
Interleukin 8 (pg/mL)												
Mean (SD)	1.68 (±0.15)	1.64 (±0.11)	1.70 (±0.16)	0.001								
Intercellular adhesion molecule (ng/mL)												
Mean (SD)	0.96 (±0.44)	0.83 (±0.33)	1.04 (±0.48)	0.0001								
Immunoglobulin A (mg/mL)												
Mean (SD)	-2.54 (±0.31)	-2.68 (±0.26)	-2.45 (±0.31)	< 0.0001								
Executive Function Score												
Mean (SD)					0.36 (±0.98)	0.38 (±1.01)	0.34 (±0.95)	0.17				
Memory Score												
Mean (SD)					0.40 (±0.92)	0.57 (±1.01)	0.26 (±0.82)	<0.0001				
Amyloid Beta												
Mean (SD)									1040.98 (±454.72)	1055.50 (±449.23)	1028.35 (±459.36)	0.18
Tau												
Mean (SD)									289.80 (±124.68)	300.90 (±139.07)	280.13 (±109.82)	0.072
PTau												
Mean (SD)									27.47 (±13.65)	28.25 (±15.08)	26.78 (±12.24)	0.36

P-values are from Wilcoxon rank sum tests for continuous variables and Fisher's exact tests for categorical variables. łIncludes self-reported Black, Asian, American Indian/Alaskan, and >1 ethnicity.

Table 3. Linear regression results for models with sex and APOE status. Only shown are the models with significant associations. P-values are for overall tests and are FDR-adjusted. All model summaries are available in Supplementary Table S1.

	ADNI MEM		**ADNI EF**		**ABETA**		**Hippocampus volume (corrected)**		**TAU**		**PTAU**	
Predictors	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*
(Intercept)	1.63 (1.12 – 2.14)		2.05 (1.47 – 2.62)		1458.67 (1247.77 – 1669.57)		11550.67 (10935.47 – 12165.88)		56.15 (-30.03 – 142.34)		4.75 (-4.89 – 14.38)	
AGE (years)	-0.02 (-0.02 – -0.01)		-0.02 (-0.03 – -0.02)		-6.19 (-9.01 – -3.36)		-65.72 (-73.95 – -57.49)		2.68 (1.54 – 3.81)		0.26 (0.13 – 0.39)	
Male (ref = Female)	-0.17 (-0.26 – -0.07)	**0.003**	-0.03 (-0.14 – 0.08)	0.73	-29.77 (-71.55 – 12.01)	0.33	482.18 (365.03 – 599.32)	**<0.0001**	-7.37 (-31.43 – 16.70)		-0.34 (-3.03 – 2.35)	
APOE status (ref = 0 alleles)		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**				
1 allele	-0.45 (-0.55 – -0.34)		-0.3 (-0.42 – -0.19)		-240.23 (-284.27 – -196.20)		-518.73 (-642.15 – -395.31)		104.14 (77.21 – 131.06)		11.73 (8.72 – 14.74)	
2 alleles	-0.69 (-0.85 – -0.53)		-0.46 (-0.64 – -0.28)		-455.95 (-521.02 – -390.88)		-946.01 (-1136.77 – -755.25)		178.88 (137.45 – 220.31)		19.81 (15.18 – 24.44)	
Interaction term										**0.001**		**0.002**
Male:1 allele									-49.76 (-85.30 – -14.22)		-5.58 (-9.55 – -1.60)	
Male:2 alleles									-101.56 (-153.97 – -49.16)		-10.78 (-16.64 – -4.92)	
Observations	1145		1144		947		1224		947		947	
R^2 / adjusted R^2	0.106 / 0.103		0.058 / 0.055		0.203 / 0.199		0.233/0.231		0.140 / 0.134		0.136 / 0.130	

Table 3. Continued

	C Reactive Protein ug/ml		**Interleukin 16 pg/ml**		**Interleukin 8 pg/m L**		**Immunoglobulin A mg/ml**		**Intercellular Adhesion Molecule 1 ng/ml**	
Predictors	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*
(Intercept)	-3.05 (-3.79 – -2.32)		0.35 (0.10 – 0.59)		1.38 (1.18 – 1.57)		-2.82 (-3.22 – -2.43)		-0.19 (-0.75 – 0.36)	
AGE (years)	0.01 (-0.00 – 0.02)		0.01 (0.00 – 0.01)		0 (0.00 – 0.01)		0 (-0.00 – 0.01)		0.01 (0.01 – 0.02)	
Male (ref = Female)	-0.12 (-0.26 – 0.01)	0.19	0.12 (0.06 – 0.18)		0.1 (0.05 – 0.15)	**0.02**	0.21 (0.14 – 0.29)	**<0.0001**	0.18 (0.07 – 0.28)	**0.003**
APOE status (ref = 0 alleles)		**0.009**				0.39		0.33		0.38
1 allele	-0.19 (-0.33 – -0.05)		0.08 (0.01 – 0.16)		0.04 (-0.02 – 0.10)		0.02 (-0.05 – 0.10)		0.09 (-0.01 – 0.20)	
2 alleles	-0.31 (-0.52 – -0.10)		0.06 (-0.04 – 0.16)		0.01 (-0.07 – 0.09)		-0.09 (-0.20 – 0.02)		0.02 (-0.13 – 0.18)	
Interaction term				**0.03**						
Male:1 allele			-0.13 (-0.22 – -0.03)							
Male:2 alleles			-0.15 (-0.28 – -0.02)							
Observations	279		279		279		279		279	
R^2 / adjusted R^2	0.058 / 0.045		0.117 / 0.098		0.092 / 0.072		0.135 / 0.122		0.107 / 0.094	

Table 4. Linear regression results for models with sex and baseline diagnosis. Only shown are the models with significant associations. P-values are for overall tests and are FDR-adjusted. All model summaries are available in Supplementary Table S2.

	ADNI MEM		**ADNI EF**		**ABETA**		**Hippocampus volume (corrected)**		**TAU**		**PTAU**	
Predictors	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*
(Intercept)	1.79 (1.45 – 2.13)		2.26 (1.80 – 2.73)		1161.57 (944.04 – 1379.10)		11424.98 (10886.11 – 11963.86)		154.16 (70.57 – 237.75)		15.72 (6.36 – 25.09)	
AGE (years)	-0.01 (-0.01 – -0.00)		-0.02 (-0.03 – -0.01)		-1.64 (-4.52 – 1.24)		-57.95 (-65.07 – -50.83)		1.25 (0.15 – 2.36)		0.1 (-0.02 – 0.23)	
Male (ref = Female)	-0.16 (-0.23 – -0.09)	**<0.0001**	-0.04 (-0.13 – 0.05)	0.56	-26.62 (-69.46 – 16.22)	0.42	516.27 (414.05 – 618.49)	**<0.0001**	-42.59 (-59.05 – -26.13)	**<0.0001**	-4.22 (-6.06 – -2.38)	**<0.0001**
Diagnosis (ref = CN)		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**
EMCI	-0.5 (-0.61 – -0.39)		-0.42 (-0.56 – -0.27)		-85.2 (-148.82 – -21.59)		-340.14 (-495.23 – -185.04)		37.85 (13.41 – 62.30)		4.22 (1.48 – 6.96)	
LMCI	-1.08 (-1.16 – -1.00)		-0.79 (-0.90 – -0.67)		-256.85 (-315.81 – -197.89)		-1049.98 (-1183.33 – -916.63)		93.34 (70.69 – 116.00)		10.58 (8.05 – 13.12)	
AD	-1.84 (-1.94 – -1.75)		-1.63 (-1.76 – -1.50)		-390.48 (-453.59 – -327.37)		-1603.56 (-1751.83 – -1455.29)		143.6 (119.35 – 167.86)		15.81 (13.10 – 18.53)	
Observations		1150		1149		947		1234		947		947
R^2 / adjusted R^2	0.589 / 0.588		0.380 / 0.377		0.168 / 0.164		0.416/ 0.414		0.164 / 0.160		0.156 / 0.152	

Table 4. Continued

	Interleukin 16 pg/ml		**Interleukin 8 pg/ml**		**Immunoglobulin A mg/ml**		**Intercellular Adhesion Molecule 1 ng/ml**	
Predictors	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*
(Intercept)	0.42 (0.18 – 0.65)		1.4 (1.20 – 1.59)		-2.9 (-3.29 – -2.50)		-0.22 (-0.77 – 0.33)	
AGE (years)	0.01 (0.00 – 0.01)		0 (0.00 – 0.01)		0 (-0.00 – 0.01)		0.01 (0.01 – 0.02)	
Male (ref = Female)	0.05 (0.01 – 0.10)	0.07	0.05 (0.01 – 0.09)	**0.03**	0.21 (0.14 – 0.29)	**<0.0001**	0.17 (0.06 – 0.27)	**0.008**
Diagnosis (ref = CN)		0.66		0.94		0.98		0.71
EMCI								
LMCI	0.01 (-0.04 – 0.06)		0.01 (-0.03 – 0.05)		0 (-0.08 – 0.09)		0.06 (-0.06 – 0.18)	
AD	-0.03 (-0.08 – 0.03)		0.01 (-0.04 – 0.06)		-0.01 (-0.11 – 0.09)		0.02 (-0.12 – 0.16)	
Observations		279		279		279		279
R^2 / adjusted R^2	0.089 / 0.075		0.059 / 0.045		0.123 / 0.111		0.101 / 0.088	

Table 5. Results of epigenetic age and epigenetic age acceleration calculation for all DNAme analyses, for both the larger DNAme cohort and the subset of samples with matched CSF biomarker data.

	DNAme Cohort				DNAme & CSF Biomarker Data Cohort			
		Sex				Sex		
	Total No. 640	**Female No. 284**	**Male No. 356**	**P-value**	**Total No. 533**	**Female No. 243**	**Male No. 290**	**P-value**
Age								
Mean (SD)	75.63 (±7.68)	74.78 (±8.03)	76.31 (±7.32)	<0.0001	75.01 (±7.61)	74.31 (±8.10)	75.61 (±7.11)	0.0019
Epigenetic Ag e (years)								
Mean (SD)	69.92 (±8.06)	67.45 (±8.15)	70.11 (±7.79)	<0.0001	68.47 (±8.17)	67.05 (±8.33)	69.72 (±7.82)	<0.0001
Epigenetic Age Acceleration (years)								
Mean (SD)	0.025 (±4.22)	-0.14 (±4.16)	0.16 (±4.26)	0.1	0.027 (±4.30)	-0.18 (±4.23)	0.20 (4.35)	0.057
Intrinsic Age Acceleration (years)								
Mean (SD)	0.026 (±4.11)	-0.19 (±4.06)	0.20 (±4.15)	0.019	0.020 (±4.18)	-0.25 (±4.14)	0.26 (±4.21)	0.021

P-values are from Wilcoxon rank sum tests for continuous variables and Fisher's exact tests for categorical variables

Table 6. Linear model for assessment of relationship of biochemical concentrations and **presence of APOE e4 risk allele** on universal epigenetic age acceleration. Intrinsic epigenetic age acceleration linear model not shown.

	Age Acceleration & CSF Biomarkers		
Predictors	*Estimates*	*CI*	*adjusted p*
(Intercept)	-0.61	-1.67 – 0.46	0.61
Male (ref = Female)	0.6	-0.11 – 1.31	0.34
Diagnosis (ref = CN)			
EMCI	0.71	-0.17 – 1.60	0.39
LMCI	0.4	-0.55 – 1.36	0.64
AD	0.53	-1.07 – 2.14	0.64
APOE risk allele			
1 or 2 alleles (ref = 0 alleles)	-0.24	-1.01 – 1.31	0.813
CSF Tau	0.0001	-0.0077 – 0.023	0.95
Observations			533
R^2 / adjusted R^2	0.0108/-0.000438		

Supplemental File

Alzheimer's disease neuropathology, inflammation and epigenetic age: do sex and APOE matter?

Paula Duarte-Guterman[1*], Amy M. Inkster[2,3*], Arianne Y. Albert[4], Cindy K. Barha[5], Wendy P. Robinson[2,3], Liisa A.M. Galea[1], on behalf of the Alzheimer's Disease Neuroimaging Initiative[δ]

1. Djavad Mowafaghian Centre for Brain Health and Department of Psychology, University of British Columbia, Vancouver, BC, Canada
2. Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada.
3. BC Children's Hospital Research Institute, Vancouver, BC, Canada
4. Women's Health Research Institute of British Columbia, Vancouver, BC, Canada
5. Djavad Mowafaghian Centre for Brain Health and Department of Physical Therapy, University of British Columbia, Vancouver, BC, Canada

Address all correspondence and requests for reprints to:
L. A. M. Galea, PhD
Djavad Mowafaghian Centre for Brain Health
2215 Wesbrook Mall
Vancouver, British Columbia
V6T 1Z3, Canada
E-mail: lgalea@psych.ubc.ca.

* Considered co-first authors.

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Table S1. Linear regression results for all variables investigated by sex and APOE status. Markers in CSF

	ADNI MEM		**ADNI EF**		**ABETA**		**Hippocampus volume (corrected)**		**Hippocampus/Intracranial volume**		**TAU**		**PTAU**		**Cortisol Cortisol ng ml**	
Predictors	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*
(Intercept)	1.63 (1.12 – 2.14)		2.05 (1.47 – 2.62)		1458.67 (1247.77 – 1669.57)		(10935.47 – 12165.88)		0.00752 (0.00709 – 0.00795)		56.15 (-30.03 – 142.34)		4.75 (-4.89 – 14.38)		-1.38 (-9.15 – 6.39)	
AGE (years)	-0.02 (-0.02 – -0.01)		-0.02 (-0.03 – -0.02)		-6.19 (-9.01 – -3.36)		-65.72 (-73.95 – -57.49)		-0.00004 (-0.0004 – -0.0003)		2.68 (1.54 – 3.81)		0.26 (0.13 – 0.39)		0.21 (0.11 – 0.32)	
Male (ref = Female)	-0.17 (-0.26 – -0.07)	**0.003**	-0.03 (-0.14 – 0.08)	0.73	-29.77 (-71.55 – 12.01)	0.33	482.18 (365.03 – 599.32)	**<0.0001**	-0.00024 (-0.00033 – -0.00016)	**<0.0001**	-7.37 (-31.43 – 16.70)		-0.34 (-3.03 – 2.35)		1.37 (-0.05 – 2.78)	0.16
APOE status (ref = 0 alleles)		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**						0.61
1 allele	-0.45 (-0.55 – -0.34)		-0.3 (-0.42 – -0.19)		-240.23 (-284.27 – -196.20)		-518.73 (-642.15 – -395.31)		-0.00031 (--0.0004 – -0.00022)		104.14 (77.21 – 131.06)		11.73 (8.72 – 14.74)		1.03 (-0.44 – 2.50)	
2 alleles	-0.69 (-0.85 – -0.53)		-0.46 (-0.64 – -0.28)		-455.95 (-521.02 – -390.88)		-946.01 (-1136.77 – -755.25)		-0.00057 (-0.00071 – --0.00044)		178.88 (137.45 – 220.31)		19.81 (15.18 – 24.44)		0.5 (-1.68 – 2.68)	
Interaction term												**0.001**		**0.002**		
Male:1 allele											-49.76 (-85.30 – -14.22)		-5.58 (-9.55 – -1.60)			
Male:2 alleles											-101.56 (-153.97 – -49.16)		-10.78 (-16.64 – -4.92)			
Observations		1145		1144		947		1224		1224		947		947		279
R^2 / adjusted R^2	0.106 / 0.103		0.058 / 0.055		0.203 / 0.199		0.233/0.231		0.191/0.189		0.140 / 0.134		0.136 / 0.130		0.086 / 0.073	

Table S1 (continued). Linear regression results for all variables investigated by sex and APOE status. Markers in CSF

	C Reactive Protein ug/ml		**CD 40 antigen ng/ml**		**Interleukin 16 pg/ml**		**Interleukin 3 ng/ml**		**Interleukin 6 receptor ng/ml**		**Interleukin 8 pg/ml**		**Immunoglobulin A mg/ml**		**Intercellular Adhesion Molecule ng/ml**	
Predictors	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*
(Intercept)	-3.05 (-3.79 – -2.32)		-1.13 (-1.29 – -0.98)		0.35 (0.10 – 0.59)		-2.96 (-3.38 – -2.54)		-0.29 (-0.48 – -0.10)		1.38 (1.18 – 1.57)		-2.82 (-3.22 – -2.43)		-0.19 (-0.75 – 0.36)	
AGE (years)	0.01 (-0.00 – 0.02)		0.01 (0.00 – 0.01)		0.01 (0.00 – 0.01)		0.01 (0.00 – 0.02)		0 (0.00 – 0.01)		0 (0.00 – 0.01)		0 (-0.00 – 0.01)		0.01 (0.01 – 0.02)	
Male (ref = Female)	-0.12 (-0.26 – 0.01)	0.19	0.01 (-0.02 – 0.04)	0.69	0.12 (0.06 – 0.18)		0.08 (0.00 – 0.16)	0.11	0.01 (-0.02 – 0.05)	0.64	0.1 (0.05 – 0.15)	**0.02**	0.21 (0.14 – 0.29	**<0.0001**	0.18 (0.07 – 0.28)	**0.003**
APOE status (ref = 0 alleles)		**0.009**		0.8				0.41		0.17		0.39		0.33		0.38
1 allele	-0.19 (-0.33 – -0.05)		0.01 (-0.02 – 0.03)		0.08 (0.01 – 0.16)		-0.03 (-0.10 – 0.05)		0.04 (0.00 – 0.08)		0.04 (-0.02 – 0.10)		0.02 (-0.05 – 0.10)		0.09 (-0.01 – 0.20)	
2 alleles	-0.31 (-0.52 – -0.10)		0.02 (-0.03 – 0.06)		0.06 (-0.04 – 0.16)		-0.1 (-0.22 – 0.02)		0.04 (-0.02 – 0.09)		0.01 (-0.07 – 0.09)		-0.09 (-0.20 – 0.02)		0.02 (-0.13 – 0.18)	
Interaction term						**0.03**										
Male:1 allele					-0.13 (-0.22 – -0.03)											
Male:2 alleles					-0.15 (-0.28 – -0.02)											
Observations		279		279		279		279		279		279		279		279
R^2 / adjusted R^2	0.058 / 0.045		0.124 / 0.111		0.117 / 0.098		0.081 / 0.068		0.045 / 0.031		0.092 / 0.072		0.135 / 0.122		0.107 / 0.094	

Table S2. Linear regression results for all variables investigated by sex and baseline diagnosis. Markers in CSF

	ADNI MEM		**ADNI EF**		**ABETA**		**Hippocampus volume (corrected)**		**Hippocampus/Intracranial volume**		**TAU**		**PTAU**		**Cortisol Cortisol ng ml**	
Predictors	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*
(Intercept)	1.79 (1.45 – 2.13)		2.26 (1.80 – 2.73)		1161.57 (944.04 – 1379.10)		11424.98 (10886.11 – 11963.86)		0.00747 (0.00710 – 0.00785)		154.16 (70.57 – 237.75)		15.72 (6.36 – 25.09)		-1.87 (-9.56 – 5.81)	
AGE (years)	-0.01 (-0.01 – -0.00)		-0.02 (-0.03 – -0.01)		-1.64 (-4.52 – 1.24)		-57.95 (-65.07 – -50.83)		-0.00003 (-0.00004 – --0.00003)		1.25 (0.15 – 2.36)		0.1 (-0.02 – 0.23)		0.22 (0.12 – 0.32)	
Male (ref = Female)	-0.16 (-0.23 – -0.09)	**<0.0001**	-0.04 (-0.13 – 0.05)	0.56	-26.62 (-69.46 – 16.22)	0.42	516.27 (414.05 – 618.49)	**<0.0001**	-0.00022 (-0.00029 – -0.00015)	**<0.0001**	-42.59 (-59.05 – -26.13)	**<0.0001**	-4.22 (-6.06 – -2.38)	**<0.0001**	1.18 (-0.25 – 2.61)	0.25
Diagnosis (ref = CN)		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**		0.5
EMCI	-0.5 (-0.61 – -0.39)		-0.42 (-0.56 – -0.27)		-85.2 (-148.82 – -21.59)		-340.14 (-495.23 – -185.04)		-0.00016 (-0.00027 – -0.00005)		37.85 (13.41 – 62.30)		4.22 (1.48 – 6.96)			
LMCI	-1.08 (-1.16 – -1.00)		-0.79 (-0.90 – -0.67)		-256.85 (-315.81 – -197.89)		-1049.98 (-1183.33 – -916.63)		-0.00073 (-0.00083 – -0.00064)		93.34 (70.69 – 116.00)		10.58 (8.05 – 13.12)		1.24 (-0.42 – 2.90)	
AD	-1.84 (-1.94 – -1.75)		-1.63 (-1.76 – -1.50)		-390.48 (-453.59 – -327.37)		-1603.56 (-1751.83 – -1455.29)		-0.00106 (-0.00116 – -0.00096)		143.6 (119.35 – 167.86)		15.81 (13.10 – 18.53)		0.24 (-1.68 – 2.16)	
Observations		1150		1149		947		1234		1234		947		947		279
R^2 / adjusted R^2	0.589 / 0.588		0.380 / 0.377		0.168 / 0.164		0.416/ 0.414		0.398 / 0.396		0.164 / 0.160		0.156 / 0.152		0.088 / 0.075	

Table S2 (continued). Linear regression results for all variables investigated by sex and baseline diagnosis. Markers in CSF

	C Reactive Protein ug/ml		**CD 40 antigen ng/ml**		**Interleukin 16 pg/ml**		**Interleukin 3 ng/ml**		**Interleukin 6.receptor ng/ml**		**Interleukin 8 pg/ml**		**Immunoglobulin A mg/ml**		**Intercellular Adhesion Molecule ng/ml**	
Predictors	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*
(Intercept)	-3.19 (-3.93 – -2.45)		-1.11 (-1.27 – -0.96)		0.42 (0.18 – 0.65)		-3 (-3.42 – -2.59)		-0.26 (-0.45 – -0.07)		1.4 (1.20 – 1.59)		-2.9 (-3.29 – -2.50)		-0.22 (-0.77 – 0.33)	
AGE (years)	0.01 (-0.00 – 0.02)		0.01 (0.00 – 0.01)		0.01 (0.00 – 0.01)		0.01 (0.00 – 0.02)		0 (0.00 – 0.01)		0 (0.00 – 0.01)		0 (-0.00 – 0.01)		0.01 (0.01 – 0.02)	
Male (ref = Female)	-0.1 (-0.24 – 0.03)	0.31	0.01 (-0.02 – 0.03)	0.81	0.05 (0.01 – 0.10)	0.07	0.08 (0.01 – 0.16)	0.11	0.01 (-0.02 – 0.05)	0.71	0.05 (0.01 – 0.09)	**0.03**	0.21 (0.14 – 0.29)	**<0.0001**	0.17 (0.06 – 0.27)	**0.008**
Diagnosis (ref = CN)		0.32		0.2		0.66		0.66		0.67		0.94		0.98		0.71
EMCI																
LMCI	-0.15 (-0.31 – 0.01)		0.01 (-0.03 – 0.04)		0.01 (-0.04 – 0.06)		-0.04 (-0.13 – 0.05)		0.01 (-0.03 – 0.05)		0.01 (-0.03 – 0.05)		0 (-0.08 – 0.09)		0.06 (-0.06 – 0.18)	
AD	-0.15 (-0.33 – 0.04)		-0.03 (-0.07 – 0.01)		-0.03 (-0.08 – 0.03)		-0.06 (-0.17 – 0.04)		-0.02 (-0.07 – 0.03)		0.01 (-0.04 – 0.06)		-0.01 (-0.11 – 0.09)		0.02 (-0.12 – 0.16)	
Observations		279		279		279		279		279		279		279		279
R^2 / adjusted R^2	0.030 / 0.016		0.138 / 0.125		0.089 / 0.075		0.077 / 0.063		0.031 / 0.017		0.059 / 0.045		0.123 / 0.111		0.101 / 0.088	

Table S3. Linear regression results for all plasma variables investigated by sex and APOE genotype

	Interleukin 18 pg/ml		**Cortisol Cortisol ng/ml**		**C Reactive Protein ug/ml**		**Intercellular Adhesion Molecule ng/ml**		**Immunoglobulin E ng/ml**		**Interleukin 8 pg/ml**	
Predictors	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*
(Intercept)	2.41 (2.25 – 2.57)		2.04 (1.92 – 2.16)		0.06 (-0.39 – 0.52)		1.91 (1.78 – 2.04)		1.66 (1.18 – 2.14)		0.79 (0.62 – 0.96)	
AGE (years)	0 (-0.00 – 0.00)		0 (-0.00 – 0.00)		0 (-0.00 – 0.01)		0 (-0.00 – 0.00)		0 (-0.01 – 0.01)		0 (0.00 – 0.01)	
Male (ref = Female)	0.06 (0.03 – 0.09)	**0.0007**	0.01 (-0.01 – 0.04)	0.45	-0.15 (-0.25 – -0.06)	**0.003**	-0.04 (-0.06 – -0.01)	**0.03**	0.25 (0.15 – 0.34)	**<0.0001**	-0.01 (-0.04 – 0.03)	0.76
APOE status (ref = 0 alleles)		0.17		0.33		**<0.0001**		0.83		0.36		0.29
1 allele	-0.03 (-0.07 – -0.00)		0.01 (-0.01 – 0.04)		-0.28 (-0.37 – -0.18)		0.01 (-0.02 – 0.04)		0.03 (-0.06 – 0.13)		-0.03 (-0.07 – 0.00)	
2 alleles	-0.04 (-0.09 – 0.01)		0.03 (-0.00 – 0.07)		-0.36 (-0.50 – -0.23)		0 (-0.04 – 0.04)		-0.1 (-0.25 – 0.04)		-0.03 (-0.09 – 0.02)	
Observations		527		527		526		527		527		527
R^2 / adjusted R^2	0.038 / 0.031		0.014 / 0.007		0.105 / 0.098		0.019 / 0.012		0.056 / 0.048		0.027 / 0.019	

Table S3 (continued). Linear regression results for all plasma variables investigated by sex and APOE genotype

	CD 40 antigen ng/ml		**Interleukin 16 pg/ml**		**Interleukin 3 ng/ml**		**Interleukin 6 receptor ng/ml**		**Immunoglobulin A mg/ml**	
Predictors	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*
(Intercept)	-0.58 (-0.68 – -0.47)		2.32 (2.18 – 2.47)		-1.71 (-1.98 – -1.45)		1.42 (1.30 – 1.55)		0.48 (0.28 – 0.69)	
AGE (years)	0.01 (0.00 – 0.01)		0 (0.00 – 0.00)		0 (-0.00 – 0.00)		0 (-0.00 – 0.00)		0 (-0.00 – 0.00)	
Male (ref = Female)	-0.01 (-0.03 – 0.01)	0.58	0.01 (-0.02 – 0.04)	0.65	0 (-0.06 – 0.05)	0.89	-0.03 (-0.05 – -0.00)	0.1	0.02 (-0.02 – 0.06)	0.61
APOE status (ref = 0 alleles)		0.61		0.69		0.7		0.4		0.65
1 allele	-0.01 (-0.03 – 0.01)		-0.02 (-0.05 – 0.01)		0.01 (-0.05 – 0.06)		0.01 (-0.01 – 0.04)		-0.01 (-0.05 – 0.03)	
2 alleles	0.01 (-0.02 – 0.04)		-0.01 (-0.06 – 0.03)		0.04 (-0.03 – 0.12)		-0.02 (-0.06 – 0.02)		-0.04 (-0.10 – 0.02)	
Observations		526		527		527		527		527
R^2 / adjusted R^2	0.133 / 0.126		0.023 / 0.015		0.002 / -0.005		0.016 / 0.008		0.009 / 0.002	

Table S4. Linear regression results for all plasma variables investigated by sex and baseline diagnosis.

	Interleukin 18 pg/ml		**Cortisol Cortisol ng/ml**		**C Reactive Protein ug/ml**		**Intercellular Adhesion Molecule ng/ml**		**Immunoglobulin E ng/ml**		**Interleukin 8 pg/ml**	
Predictors	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*
(Intercept)	2.33 (2.17 – 2.49)		2.08 (1.96 – 2.20)		-0.07 (-0.56 – 0.41)		1.92 (1.78 – 2.05)		1.6 (1.11 – 2.09)		0.78 (0.61 – 0.96)	
AGE (years)	0 (-0.00 – 0.00)		0 (-0.00 – 0.00)		0.01 (0.00 – 0.01)		0 (-0.00 – 0.00)		0 (-0.01 – 0.01)		0 (0.00 – 0.01)	
Male (ref = Female)	0.06 (0.03 – 0.09)	**0.001**	0.02 (-0.01 – 0.04)	0.35	-0.14 (-0.24 – -0.05)	**0.01**	-0.03 (-0.06 – -0.01)	0.05	0.25 (0.15 – 0.34)	**<0.0001**	0 (-0.04 – 0.03)	0.89
Diagnosis (ref = CN)		0.56		**0.005**		**0.04**		0.18		0.91		0.28
LMCI	0.04 (-0.01 – 0.10)		-0.02 (-0.07 – 0.02)		-0.26 (-0.44 – -0.09)		-0.01 (-0.06 – 0.03)		-0.01 (-0.18 – 0.17)		-0.04 (-0.10 – 0.02)	
AD	0.03 (-0.03 – 0.10)		0.03 (-0.02 – 0.08)		-0.22 (-0.41 – -0.03)		0.02 (-0.03 – 0.08)		-0.04 (-0.23 – 0.16)		0 (-0.07 – 0.06)	
Observations		527		527		526		527		527		527
R^2 / adjusted R^2	0.032 / 0.025		0.034 / 0.026		0.043 / 0.036		0.029 / 0.021		0.050 / 0.043		0.027 / 0.020	

Table S4 (continued). Linear regression results for all plasma variables investigated by sex and baseline diagnosis.

	CD 40 antigen ng/ml		**Interleukin 16 pg/ml**		**Interleukin 3 ng/ml**		**Interleukin 6 receptor ng/ml**		**Immunoglobulin A mg/ml**	
Predictors	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*	*Estimates (CI)*	*adjusted p*
(Intercept)	-0.56 (-0.67 – -0.46)		2.38 (2.23 – 2.53)		-1.62 (-1.89 – -1.35)		1.44 (1.32 – 1.56)		0.42 (0.22 – 0.63)	
AGE (years)	0.01 (0.00 – 0.01)		0 (0.00 – 0.00)		0 (-0.00 – 0.00)		0 (-0.00 – 0.00)		0 (-0.00 – 0.00)	
Male (ref = Female)	-0.01 (-0.03 – 0.01)	0.62	0.01 (-0.02 – 0.04)	0.58	-0.01 (-0.06 – 0.05)	0.91	-0.02 (-0.05 – -0.00)	0.14	0.02 (-0.02 – 0.06)	0.61
Diagnosis (ref = CN)		0.06		0.06		0.1		0.56		0.84
LMCI	-0.02 (-0.05 – 0.02)		-0.08 (-0.13 – -0.02)		-0.04 (-0.14 – 0.06)		-0.03 (-0.08 – 0.01)		0.03 (-0.05 – 0.10)	
AD	0.02 (-0.02 – 0.06)		-0.08 (-0.14 – -0.02)		-0.12 (-0.22 – -0.01)		-0.03 (-0.08 – 0.02)		0.02 (-0.07 – 0.10)	
Observations		526		527		527		527		527
R^2 / adjusted R^2	0.143 / 0.137		0.036 / 0.029		0.013 / 0.006		0.014 / 0.006		0.008 / -0.000	

Supplementary Table S5. Correlation matrix of biochemical variables for those ADNI samples with available DNAme and biochemical data (n=533). Correlation coefficients are Spearman's rho.

	Amyloid Beta	p value	Tau	p value	pTau	p value
Amyloid Beta	1.00	<0.0001	-0.18	<0.0001	-0.27	<0.0001
Tau	-0.18	<0.0001	1.00	<0.0001	0.98	<0.0001
pTau	-0.27	<0.0001	0.98	<0.0001	1.00	<0.0001