Sex and APOE genotype influence AD neuropathology but not epigenetic age across diagnosis

Paula Duarte-Guterman[1*], Amy M. Inkster[2,3*], Arianne Y. Albert[4], Cindy 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

Introduction: Alzheimer's disease (AD) disproportionately affects females. We determined whether physiological biomarkers (neuroplasticity, immune, stress, epigenetic) explain why females are more susceptible to AD than males using the Alzheimer's Disease Neuroimaging Initiative (ADNI) database.

Methods: Using the complete ADNI cohort, we analysed the effect of sex and APOE genotype (number of ε4 alleles) and sex and diagnosis (cognitively normal (CN), mild cognitive impairment (MCI), AD) on (1) AD related endpoints: memory scores, executive function scores, hippocampal volume, cerebrospinal fluid (CSF) amyloid beta, tau and p-tau; (2) markers of the immune system (interleukins, C-reactive protein, and immunoglobulins), neuroplasticity (intercellular adhesion molecule, ICAM1), and stress (cortisol); and (3) epigenetic age.

Results: Females had higher levels of tau and p-tau compared to males and increasing alleles of APOEε4 disproportionately increased tau and p-tau compared to males. Females had larger hippocampal volume (corrected with intracranial volume) and better memory scores (that include verbal memory) than males, regardless of APOE genotype and diagnosis. There were also sex differences in biomarkers with females having higher levels of plasma C-reactive protein and lower levels of CSF IL-8, IL-16, immunoglobulin A, and ICAM1. We did not observe an association between sex, diagnosis, or APOE genotype and blood epigenetic age acceleration or intrinsic epigenetic age acceleration.

Conclusion: In females tau pathology was increased but memory scores were higher and corrected hippocampal volume were larger compared to males suggesting females have a reserve against brain damage that delays either the onset of cognitive decline or diagnosis. In this ADNI cohort more males than females were diagnosed with MCI but with no significant difference in

AD diagnosis, although more females presented with AD, suggesting the progression from CN, MCI to AD may be sex-specific. We found sex differences in immune biomarkers indicating that the underlying physiology may participate in differential aging with and without a diagnosis of AD or MCI between the sexes.

Keywords: Sex differences, inflammation, epigenetic age, hippocampus

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease characterized by severe cognitive decline (Alzheimer's Association, 2017). Modifiable risk factors associated with AD include stress (Caruso et al., 2018), sociocultural or lifestyle factors (e.g., education, marital status, exercise), and conditions (diabetes, obesity, and cardiovascular disease; Baumgart et al., 2015; Nebel et al., 2018; Xu et al., 2015). Non-modifiable risk factors include age, biological sex, and APOE genotype (Riedel et al., 2016). 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 (reviewed by Ferretti et al., 2018; Mielke et al., 2014; Nebel et al., 2018). Nevertheless, regardless of prevalence, females show greater neuropathology (brain atrophy, neurofibrillary tangles) and cognitive decline with AD than males in both Europe and the United States (Ardekani et al., 2016; Barnes et al., 2005; Holland et al., 2013; Hua et al., 2010; Irvine et al., 2012; Koran and Hohman, 2017; Lin et al., 2015).

The hippocampus is one of the first brain areas to show atrophy with AD (Apostolova et al., 2006; Jack et al., 2000; Kidron et al., 1997) and hippocampal atrophy correlates with cognitive decline (Petersen et al., 2000) and AD pathology (neurofibrillary tangles; Jack et al., 2002). Previous studies using the Alzheimer's Disease Neuroimaging Initiative (ADNI) indicate that females have greater atrophy rates and cognitive decline than males with AD (Holland et al., 2013; Hua et al., 2010; Lin et al., 2015). However, there is limited research into the role of sex in the possible mechanisms underlying AD. In addition, few studies have examined the interaction of genetic polymorphisms and biological sex in AD. The ε4 allele of the APOE gene is a well-known genetic risk factor of AD (Corder et al., 1993) and is associated with accumulation of amyloid beta protein (Ossenkoppele et al., 2015). In females between 65 and 75 years, one allele of ε4 increases the risk of AD by 4-fold relative to males, indicating that the APOE genotype affects males and females differently (meta-analysis by Neu et al., 2017). 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.

Chronic inflammation is a hallmark of AD, as evidenced by increased expression of proinflammatory cytokines in the brains of AD patients which can exacerbate AD pathology (Heppner et al., 2015; Kinney et al., 2018; Swardfager et al., 2010). There are sex differences in immune responses (Klein and Flanagan, 2016) which can affect neuroplasticity (Dantzer, 2018; de Miranda et al., 2017) and interact with stress (Dantzer, 2018), but it is not known how these may be related to sex differences in AD. 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, the first objective of this study was to investigate potential

physiological biomarkers (neuroplasticity, immune, stress) that may explain sex differences in AD and in people at risk for AD using the ADNI database.

Aging biomarkers also include epigenetic alterations, and these have been associated with a variety of pathologies and adverse health conditions, including normal cognitive aging and neurodegenerative phenotypes such as AD (Hannum et al., 2013; Horvath, 2013; Levine et al., 2015; Yokoyama et al., 2017). Recently, molecular biomarkers of aging known as "epigenetic clocks" have been developed based on DNA methylation signatures (Hannum et al., 2013; Horvath, 2013). 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 (Horvath, 2013). 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 (Gale et al., 2018; Horvath, 2013; Horvath et al., 2015; Horvath and Ritz, 2015), while negative deviations of epigenetic age from chronological age (negative epigenetic age acceleration) have been associated with high life-expectancy populations and memory retention (Degerman et al., 2017; McEwen et al., 2017). In AD, epigenetic age acceleration of the frontal cortex was associated with amyloid load, neuritic plates, and cognitive decline (Levine et al., 2015). 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 (Braun et al., 2019), 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 (Horvath et al., 2016); 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, neuropathological markers of AD and the potential underlying physiological mechanisms (neuroplasticity, immune, stress) and how these may be affected by APOE genotype (number of ε4 alleles), and secondly by dementia status (cognitively healthy (CN), mild cognitive impairment (MCI), AD). Our third objective was to investigate epigenetic age in peripheral tissue of CN, MCI and AD participants, and to study the relationship between sex, APOE genotype, dementia status, and epigenetic age acceleration.

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 that had a baseline diagnosis in the ADNI database (total n = 1,460, n= 630 females, n=830 males). 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 Crane et al., 2012; Gibbons et al., 2012), hippocampal volume (mm^3), cerebrospinal fluid (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 (Gibbons et al., 2012). The composite memory score included Rey Auditory Verbal Learning Test, AD Assessment Schedule - Cognition, Mini-Mental State Examination, and Logical Memory data (Crane et al., 2012). A small subset of participants also had inflammatory markers measured in CSF (N = 279), and plasma (N = 527) listed in Table 2A. Hippocampal volume was divided by intracranial volume to correct for differences in brain size, as sex differences in hippocampal volume are influence by intracranial volume (Lotze et al., 2019; Tan et al., 2016) and is presented as a ratio.

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 and cognitive ability, corrected hippocampal volume,

and biomarkers. All models included age as a covariate. To test the main question, 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 (Benjamini and Hochberg, 1995). All regression analyses were carried out in R v3.5.1 (R Core Team 2018).

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; Vasanthakumar et al., 2017) with CN, MCI and AD diagnosis. DNAme IDAT files were read into R v3.5.1 (R Core Team, 2018) 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) (Aryee et al., 2014; Fortin et al., 2017). 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 (Aryee et al., 2014; Fortin et al., 2017), the remaining samples were background normalized and dye-bias adjusted with normal exponential out-of-band ("noob") normalization (Triche et al., 2013). DNAme data were converted to beta values and biological sex for all samples was confirmed by clustering samples on all DNAme 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/ (Horvath, 2013; Teschendorff et al., 2013). The missing DNAme values at these CpG sites can also be imputed based on the k-nearest neighbors method. We observed a very 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; we therefore chose to remove missing probes (Fiorito et al., 2017; McEwen et al., 2018).

Prior to statistical analyses we removed all technical replicates. Epigenetic age acceleration was calculated as the residual of epigenetic age regressed on chronological age and technical/batch covariates, including 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. (Chen et al., 2016) 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 methylation data with the Houseman algorithm (Houseman et al., 2012). For participants who contributed more than one blood DNAme sample within the 2-year collection period, we determined that longitudinal data collected within the median 3.6-year error of the epigenetic clock could not be meaningfully evaluated, and therefore calculated mean epigenetic age acceleration measures 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, dementia diagnosis, or APOE genotype, we used unbalanced two-way ANOVA designs. With CSF biomarker (amyloid beta, tau and p-tau) data available from the ADNI repository for a smaller subset of participants with matched EPIC DNAme data, (n=533, see Table 2C) we used linear regression to test whether APOEε4 genotype, amyloid beta, tau, p-tau, dementia diagnosis, or sex were significantly associated with epigenetic age acceleration.

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 ($P<0.05$). There were more females with a baseline diagnosis of AD compared to males (23.7% compared to 21.7%, unadjusted $P = 0.41$), although not significantly, and more females were cognitively normal than males (26.7% compared to 20.8%, unadjusted $P = 0.01$). 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 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, females had a smaller uncorrected hippocampal volume but larger corrected hippocampal volume, greater CSF amyloid beta, tau and p-tau, and higher memory function z-scores than males (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, interleukin-3, interleukin 8, interleukin-16, immunoglobulin A, and intercellular adhesion molecule 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 (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. 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 and D).

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 and larger corrected hippocampal volume across all APOE genotypes (Fig 1 E and F). Lower memory z-scores were associated with increasing number of APOEε4 alleles in both sexes. Similarly, corrected hippocampus volume was significantly lower with increasing number of APOEε4 alleles in both sexes. Increasing APOEε4 alleles was also associated with lower executive function z-scores, lower amyloid beta, and lower C-reactive protein (Table 3; Fig 1 G-I), however there was no additional association of these variables with sex. Finally, results were similar for biomarkers in plasma (Supplementary Table S3).

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 scores, larger corrected hippocampus volume, and higher tau and p-tau compared to males, irrespective of diagnosis. As expected, increasing

282 severity of diagnosis was associated with lower memory and executive function scores, smaller
283 corrected hippocampus volume, and higher CSF tau and p-tau irrespective of sex (Fig 2 A-D).
284 We found that although females had higher CSF levels of interleukin 16 (IL-16), and
285 lower levels of interleukin 8 (IL-8), immunoglobulin A (IgA), and intercellular adhesion
286 molecule 1 (ICAM1), controlling for age, compared to males, there was no association between
287 these variables and diagnosis (Fig 2 E-H). Finally, there were associations between diagnosis and
288 executive function z-scores, and amyloid beta, controlling for age, but not between these
289 variables and sex (Fig 2 I and J).
290 The results for biomarkers and inflammatory markers in plasma were similar
291 (Supplementary Table S4), with the exception of a significant relationship between plasma C-
292 reactive protein (CRP) and sex (adjusted p=0.03), and also between plasma cortisol and baseline
293 diagnosis (adjusted P=0.01; Fig 2 K and L). Males have lower levels of CRP compared to
294 females and we observed a trend between diagnosis and CRP levels in plasma with lower CRP
295 levels in late MCI and AD (adjusted P=0.08). Plasma cortisol was lower in late MCI compared
296 to CN but higher in AD compared to CN. In summary, although we detected associations
297 between sex and diagnosis and various parameters, we did not find evidence for a clear sex and
298 diagnosis interaction.
299
300 *Epigenetic age, sex, dementia diagnosis, and AD biomarkers*
301 We investigated the hypothesis that sex and dementia diagnosis affect epigenetic age
302 acceleration in blood samples of ADNI participants (see Table 5).
303 Epigenetic age acceleration was not associated with sex, dementia diagnosis (CN, EMCI,
304 LMCI, and AD), or the interaction of sex and diagnosis after multiple test correction (Figure 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 phosphorylated 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, amyloid beta concentration, tau and p-tau concentration on epigenetic age acceleration and intrinsic epigenetic age acceleration with linear regression. None of these variables was 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 scores have been demonstrated to be independently predictive of the transition from mild cognitive impairment to a formal diagnosis of Alzheimer's disease (Gibbons et al. 2012, Gale et al. 2013). By a two-way unbalanced ANOVA models investigating the effect of sex and memory score 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, hippocampal volume (corrected by intracranial 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 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, females had larger corrected hippocampal volume and better memory scores which may contribute to their delayed diagnosis (Sundermann et al., 2017). 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, slightly more females presented with a diagnosis of AD compared to males, whereas significantly more males presented with a diagnosis of MCI supporting the prevalence observed in bigger populations (Winblad et al., 2016; Mielke et al., 2014). Previous work has demonstrated sex differences in rates of AD and symptoms of AD (reviewed in Ferretti et al., 2018; Mielke et al., 2014; Nebel et al., 2018), 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.

351

352 *Females show greater tau neuropathology disproportionately affected by APOE genotype*

353 In the present study, we found that females have significantly higher baseline tau and p-
354 tau levels in CSF than males and these are indicative of the formation of neurofibrillary tangles
355 and AD pathology (Blennow et al., 2015; Henriques et al., 2018). This is in agreement with a
356 recent ADNI study (Sundermann et al., 2018; but see an earlier ADNI study Holland et al.,
357 2013) and with animal models (Lewis et al., 2001). Intriguingly, we also found that levels of tau
358 and p-tau were disproportionately elevated with APOEε4 allele expression in females compared
359 to males. Previous studies indicate that females with the APOEε4 allele are at a greater risk for
360 developing AD than are males with this allele (Altmann et al., 2014), and sex differences in tau
361 and p-tau may be one underlying mechanism by which this occurs. In females (65-75 years of
362 age) one allele of ε4 increases the risk of AD by 4-fold relative to males, indicating that genotype
363 may affect females differently (Neu et al., 2017). Levels of CSF tau are hypothesized to increase
364 after CSF amyloid beta declines and amyloid beta aggregates and deposits in the brain (Blennow
365 et al., 2015). However, in this study although we found sex differences in CSF tau and p-tau
366 levels, no significant differences were seen in CSF amyloid beta after controlling for age (see
367 below) indicating that the pathway may be different in females compared to males or that the
368 timeline of tau and amyloid beta deposition may not be consistent.

369 In this ADNI cohort, more females presented with a diagnosis of AD compared to males.
370 Although the ADNI cohort is relatively small, this result supports the prevalence observed in
371 bigger populations (Winblad et al., 2016). Together with the disproportionate effect of APOE
372 genotype on tau-related pathology it supports the idea that females have a higher burden of the
373 disease. On the other hand, more males presented with a diagnosis of MCI and this is in line with

the research that males are more likely to be diagnosed with MCI compared to females (Mielke et al., 2014). Females progress faster from MCI to AD (Lin et al., 2015) and 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 for intracranial volume. 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 (Apostolova et al., 2006; Buckner, 2004; Ewers et al., 2012; Jack et al., 2000; Li et al., 2016; Mungas et al., 2010; Petersen et al., 2000; Pievani et al., 2011; Shi et al., 2014). Surprisingly, although females have higher levels of tau and p-tau, they presented with larger corrected hippocampal volume and better memory and executive function scores than males, regardless of diagnosis and APOE genotype. 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 (Tan et al., 2016), a finding that is supported by the current study. In a number of studies, including the present study, males have a larger hippocampus without correcting for intracranial volume (Cavedo et al., 2018; Jack et al., 2015; Murphy et al., 1996; Ritchie et al., 2018; Sohn et al., 2018; Sundermann et al., 2018; Tan et al., 2016). However after correcting for intracranial volume, either the sex difference disappears (Cavedo et al., 2018; Ritchie et al., 2018; Tan et al., 2016) or females have larger corrected hippocampal volume (this study; Jack et al., 2015; Murphy et al., 1996; Sohn et al., 2018; Sundermann et al., 2018). Regardless of hippocampal volume, volume loss is greater in

aging females (Ardekani et al., 2016; Koran et al., 2017; Murphy et al., 1996) and in females with one or two APOEε4 alleles (Fleisher et al., 2005). 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 (Sundermann et al., 2018). In addition, also using the ADNI database, Koran et al. (2017) 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 that in addition to larger corrected hippocampal volume, females also 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 (Jack et al., 2015), and in MCI and AD ADNI cohorts compared to males (Sundermann et al., 2018, 2016). Here we used the ADNI memory score developed by Crane et al. (2012) 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.(2018) 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 and corrected hippocampal volume were larger compared to males suggesting females have a reserve against brain damage that delays either the onset of cognitive decline (Stern, 2002) or diagnosis (Sundermann et al., 2017). However, once cognitive decline begins, females show higher rates of declines compared to males (this was observed by Buckley et al., 2018; Holland et al., 2013; Hua et al., 2010 using the ADNI database) 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 (Henriques et al., 2018). These findings are consistent with data from studies in AD patients (Buckley et al., 2018) and in cognitively normal individuals (Jack et al., 2015). 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 (Sundermann et al., 2018) or in cognitively normal adults in the anterior cingulate (Cavedo et al., 2018). In this study, we used CSF amyloid beta data which detects abnormal amyloid deposition earlier than amyloid beta by PET (reviewed in Blennow et al., 2015). Thus, taken together, sex differences in amyloid beta may be detected in

443 specific brain regions and later in the disease, although more research is needed investigating sex
444 differences in AD biomarkers.
445
446 *Females have higher CRP levels but lower cytokine and immunoglobulin levels compared to*
447 *males*
448 In this study, we investigated whether sex interacted with APOE genotype or dementia
449 diagnosis to influence inflammatory, neurotrophic and neuroplasticity markers. We found that
450 plasma CRP, a widely used inflammatory and cardiovascular marker (Koenig et al., 1999; Ridker
451 et al., 1998), was affected by sex and APOE genotype. Females, regardless of diagnosis or
452 APOE genotype, had significantly higher plasma CRP relative to males, consistent with findings
453 in healthy individuals (Khera et al., 2005). Higher levels of peripheral CRP may suggest higher
454 inflammation in females, which is associated with an increased risk in all-cause dementia
455 (Koyama et al., 2013). In contrast, APOEε4 genotype decreased circulating CRP levels,
456 consistent with previous research in large population studies (Hubacek et al., 2010; Yun et al.,
457 2015). Recent meta-analyses, without regard to sex, did not find differences in peripheral levels
458 of CRP in AD compared to control patients (Gong et al., 2016; Ng et al., 2018). However, in
459 patients with mild and moderate dementia only, CRP levels were lower compared to the healthy
460 control group (Gong et al., 2016). To our knowledge, no other study has examined sex
461 differences in CRP in relation to AD.
462 We also found that CSF IL-16 was affected by sex and APOE genotype. CSF IL-16
463 levels were lower in females with no APOEε4 alleles compared to males, but with increasing
464 number of ε4 alleles, no sex differences were detected. IL-16 has been implicated in AD (Rosa et
465 al., 2006) and IL-16 levels decrease with disease severity (analysis without regard to sex; Motta

et al., 2007). 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 (Ponthieux et al., 2003). ICAM1 is a type of adhesion molecule associated with microvascular endothelial activation (Zenaro et al., 2017) and plasma ICAM1 levels (but not CSF levels; Nielsen et al 2007) were higher in patients with AD (Huang et al 2015; Nielsen et al 2007; Rentzos et al 2004). 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 (Walters et al., 2016). Increased expression of pro-inflammatory cytokines contributes to neuronal loss, while anti-inflammatory effects contribute to amyloid beta clearance (Heneka et al., 2015). In AD mouse models, some pro-inflammatory mechanisms reduced plaque pathology, while anti-inflammatory cytokines increased amyloid beta deposition (Chakrabarty et al., 2012, 2011, 2010a, 2010b; Ghosh et al., 2013; Shaftel et al., 2007). It has been suggested that there are beneficial pro-inflammatory mechanisms and detrimental anti-inflammatory mechanisms in AD (Heneka et al., 2015). 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. 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 (Levine et al., 2015). While brain-blood methylation profiles are reasonably correlated (r=0.86) (Braun et al., 2019), 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 (Levine et al., 2015). 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 (GTEx Project, 2018) further support this hypothesis.

Intriguingly, epigenetic age was observed to be lower on average than chronological age (see Table 5). 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

512 calculate epigenetic age via this method do not exist on the EPIC array. Two previous studies
513 investigated the application of Horvath's epigenetic clock to EPIC data with conflicting results
514 (Dhingra et al., 2019; McEwen et al., 2018), the largest issue being chronic underestimation of
515 epigenetic age due to the positive linear regression coefficients associated with the missing
516 probes(Dhingra et al., 2019). Both imputing and removing the missing probes from the array
517 resulted in a chronic underprediction of epigenetic age with Horvath's clock, suggesting that this
518 is likely an artefact of the array platform and probe-set rather than the method chosen to deal
519 with missing values, although it is possible that an adjustment factor could be devised to more
520 accurately apply Horvath's clock to EPIC data. In future explorations of epigenetic age with
521 EPIC DNAme array data this should be considered, as there are other epigenetic age predictors
522 available that have been trained on EPIC data such as the PhenoAge and GrimAge clocks,
523 although these tools have limitations as well; for example, both PhenoAge and GrimAge were
524 trained only on blood DNAme data, as compared to the original pan-tissue epigenetic clock, and
525 therefore may have limited applicability and relevance in other tissues (Levine et al., 2018; Lu et
526 al., 2019).
527
528 **Limitations**
529 The ADNI cohort is not ethnically or socioeconomically diverse, being mostly composed
530 of white (only 12 individuals were not-white) and highly educated individuals (average 15.69
531 years of education). As incidence, prevalence, and age of onset of AD varies by ethnicity
532 (Hispanics, Fitten et al., 2014; Mayeda et al., 2016; African-Americans, Steenland et al., 2016)
533 and education (Sharp and Gatz, 2011), our conclusions may not apply to more ethnically and
534 socially diverse populations. In addition to sex, it is possible the underlying mechanisms of AD

535 are different depending on ethnicity. Finally, the ADNI biomarker data set has a low sample size
536 (279 total), especially when taking into account diagnosis, sex and APOE genotype. Small
537 sample size is also a limitation of the epigenetic analyses presented. Even in the larger 640-
538 participant cohort, only 37 participants (5.78%) had an AD diagnosis, so statistical analyses were
539 underpowered to detect subtle differences by diagnosis group. Additionally other pathologies in
540 these participants, such as cancer, cardiovascular disease, smoking status, or obesity may have
541 influenced AD neuropathology, biomarkers and epigenomes and limited our interpretations.
542
543 **Conclusion**
544 As expected, more females presented with a diagnosis of AD whereas more males
545 presented with MCI diagnosis compared to the opposite sex. AD biomarkers (CSF tau and p-tau
546 but not amyloid beta) were disproportionately affected by APOE genotype in females compared
547 to males supporting the idea that females share a higher burden of the disease. Interestingly,
548 although females in this cohort had elevated AD biomarkers, they also had larger corrected
549 hippocampal volume and higher memory function scores compared to males, regardless of
550 APOE genotype and dementia diagnosis. Therefore, it is possible that females may have a
551 reserve that protects the brain from damage to delay cognitive decline or delay diagnosis.
552 Finally, we found that females had lower cytokine and immunoglobulin levels but higher CRP
553 levels compared to males. Together our work suggests that that the underlying physiology of
554 aging and AD may be sex-specific.
555
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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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Table 1. Demographic and clinical information for all participants and subdivided by sex. Biomarkers for AD are from cerebrospinal fluid. P-values after adjusting for age are presented here for easier comparison and are taken from the linear model of sex and diagnosis (see Table 3 for 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					
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 (corrected)					
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)	15.96 (±2.52)	15.84 (±2.54)	16.04 (±2.50)	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	n/a	n/a	n/a		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. ŀIncludes 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. All model summaries are available in Supplementary Table S1.

	ADNI MEM			ADNI EF			ABETA			Hippocampus/Intracranial volume			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		0.00752	0.00709 – 0.00795		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		-0.00004	-0.0004 – -0.0003		2.68	1.54 – 3.81		0.26	0.13 – 0.39	
Male (ref = Female)	-0.17	-0.26 – -0.07	**0.002**	-0.03	-0.14 – 0.08	0.68	-29.77	-71.55 – 12.01	0.28	-0.00024	-0.00033 – -0.00016	**<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		-0.00031	--0.0004 – -0.00022		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		-0.00057	-0.00071 – --0.00044		178.88	137.45 – 220.31		19.81	15.18 – 24.44	
Interaction term															**0.0008**			**0.001**
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			0.191/0.189			0.140 / 0.134			0.136 / 0.130		

Table 3. Continued

	C Reactive Protein ug/ml			Interleukin 16 pg/ml			Interleukin 8.IL 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	0.15	0.12	0.06 – 0.18		0.1	0.05 – 0.15	**0.01**	0.21	0.14 – 0.29	**<0.0001**	0.18	0.07 – 0.28	**0.002**
APOE status (ref = 0 alleles)			**0.007**						0.33			0.27			0.31
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						**0.02**									
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/Intracranial volume**			**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		0.00747	0.00710 – 0.00785		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		-0.00003	-0.00004 – --0.00003		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	0.53	-26.62	-69.46 – 16.22	0.38	-0.00022	-0.00029 – -0.00015	**<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		-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		-0.00073	-0.00083 – -0.00064		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		-0.00106	-0.00116 – -0.00096		143.6	119.35 – 167.86		15.81	13.10 – 18.53	
Observations	1150			1149			947			1234			947			947		
R^2 / adjusted R^2	0.565 / 0.563			0.280 / 0.277			0.168 / 0.164			0.398 / 0.396			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	0.06	0.05	0.01 – 0.09	**0.02**	0.21	0.14 – 0.29	**<0.0001**	0.17	0.06 – 0.27	**0.006**
Diagnosis (ref = CN)			0.64			0.96			0.98			0.67
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 APOE genotype on universal epigenetic age acceleration. Intrinsic epigenetic age acceleration linear model not shown.

	Age Acceleration & CSF Biomarkers		
Predictors	*Estimates*	*CI*	*adjusted p*
(Intercept)	-1.18	-2.80 – 0.45	0.517
Male (ref = Female)	0.65	-0.61 – 1.37	0.448
Diagnosis (ref = CN)			
EMCI	0.77	-0.12 – 1.65	0.09
LMCI	0.47	-0.51 – 1.45	0.569
AD	0.54	-1.10 – 2.19	0.738
APOE status (ref = 0 alleles)			
1 allele	-0.031	-0.09 –0.84	0.945
2 alleles	-0.3	-1.75 – 1.14	0.813
CSF Amyloid Beta	0.00018	-0.00083 – 0.0011	0.813
CSF Tau	0.0078	-0.0077 – 0.023	0.569
CSF PTau	-0.072	-0.22 – 0.072	0.569
Observations			533
R^2 / adjusted R^2	0.0143/-0.00262		

Figure 1

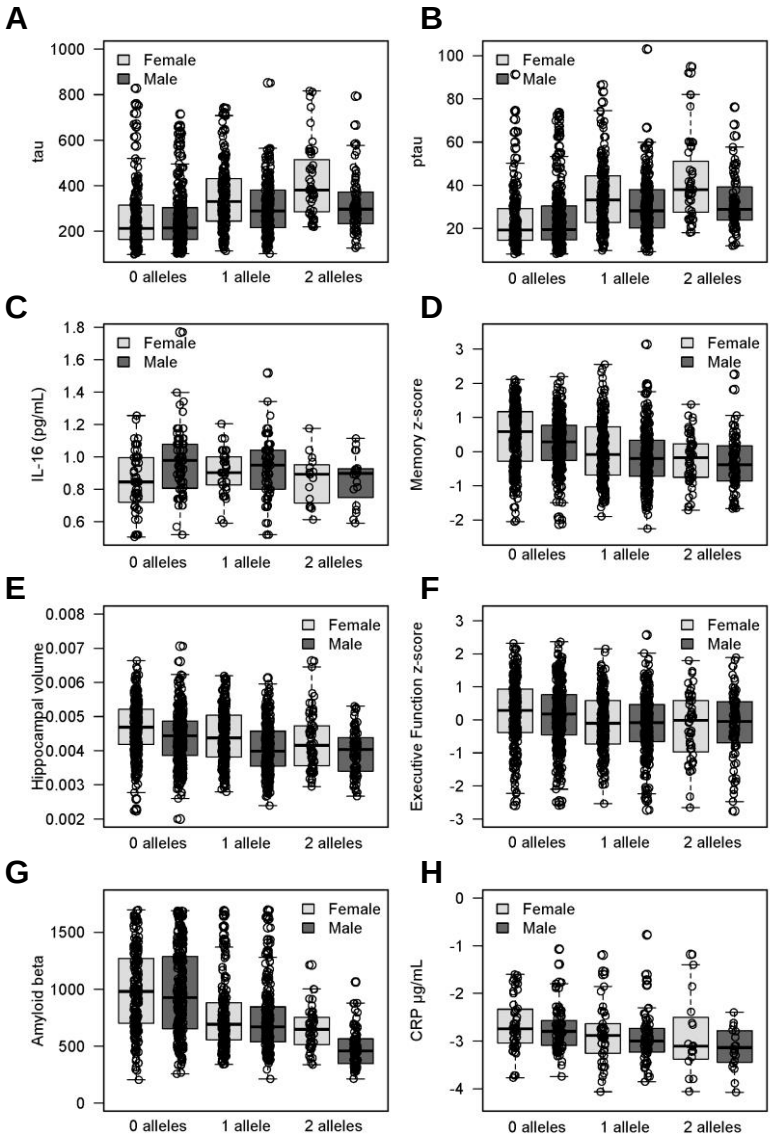

A
tau
Female
Male
0 alleles
1 allele
2 alleles
B
ptau
C
IL-16 (pg/mL)
D
Memory z-score
E
Hippocampal volume
F
Executive Function z-score
G
Amyloid beta
H
CRP µg/mL

Figure 2

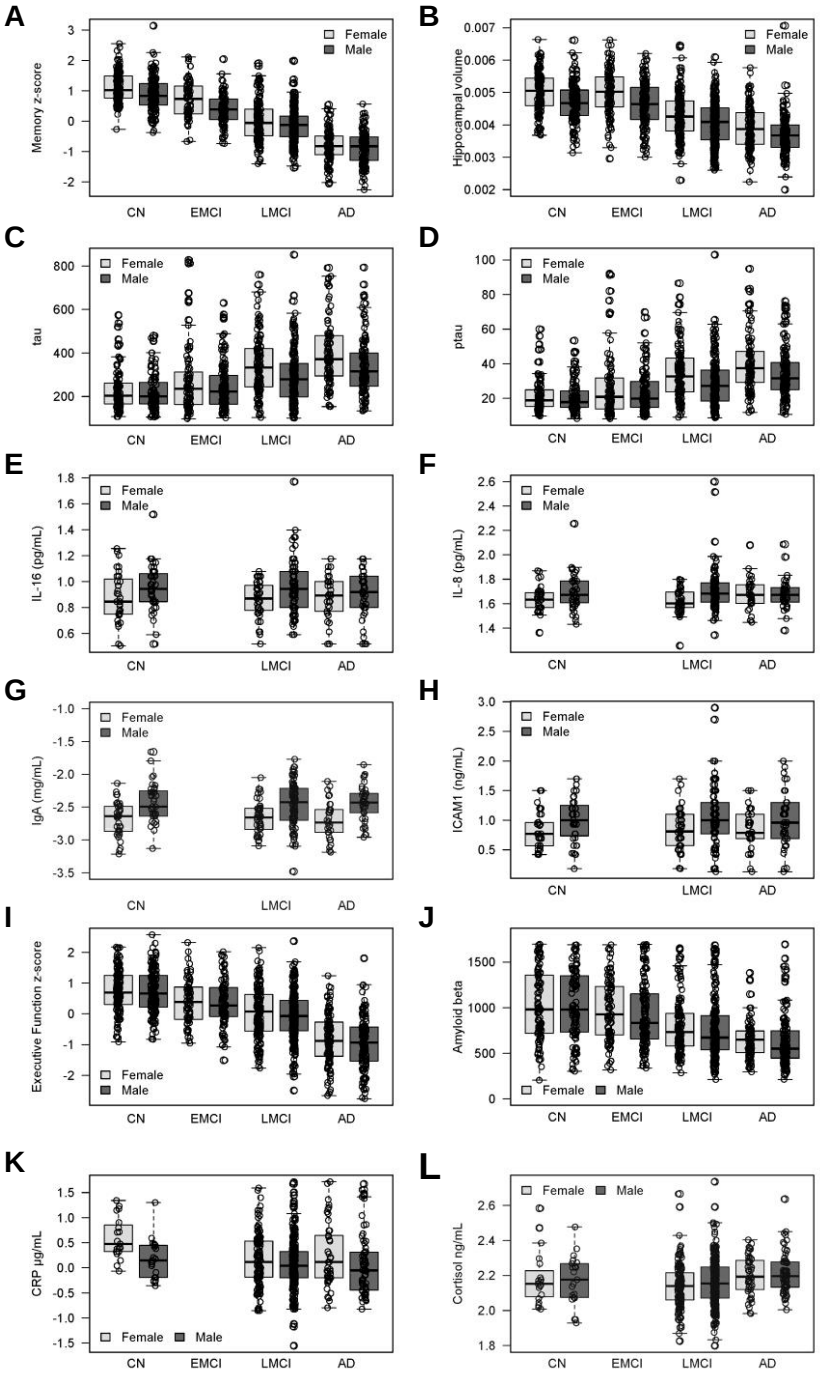
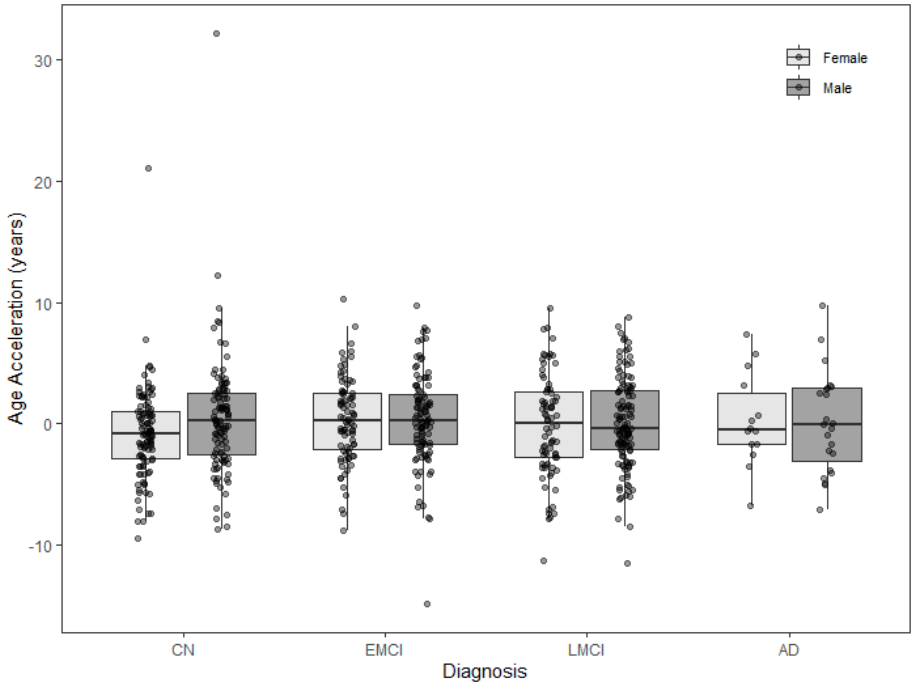

A
Memory z-score
Female
Male
CN
EMCI
LMCI
AD
B
Hippocampal volume
C
tau
D
ptau
E
IL-16 (pg/mL)
F
IL-8 (pg/mL)
G
IgA (mg/mL)
H
ICAM1 (ng/mL)
I
Executive Function z-score
J
Amyloid beta
K
CRP µg/mL
L
Cortisol ng/mL

Figure 3


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

Supplemental File

Sex and APOE genotype influence AD neuropathology but not epigenetic age across diagnosis

Paula Duarte-Guterman[1*], Amy M. Inkster[2,3*], Arianne Y. Albert[4], Cindy 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

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

	ADNI MEM			ADNI EF			ABETA			Hippocampus/Intracranial volume			TAU			PTAU			Cortisol Cortisol ng ml			C Reactive Protein ug/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		0.00752	0.00709 – 0.00795		56.15	-30.03 – 142.34		4.75	-4.89 – 14.38		-1.38	-9.15 – 6.39		-3.05	-3.79 – -2.32	
AGE (years)	-0.02	-0.02 – -0.01		-0.02	-0.03 – -0.02		-6.19	-9.01 – -3.36		-0.00004	-0.0004 – -0.0003		2.68	1.54 – 3.81		0.26	0.13 – 0.39		0.21	0.11 – 0.32		0.01	-0.00 – 0.02	
Male (ref = Female)	-0.17	-0.26 – -0.07	**0.002**	-0.03	-0.14 – 0.08	0.68	-29.77	-71.55 – 12.01	0.28	-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	0.12	-0.12	-0.26 – 0.01	0.15
APOE status (ref = 0 alleles)			**<0.0001**			**<0.0001**			**<0.0001**			**<0.0001**									0.52			**0.007**
1 allele	-0.45	-0.55 – -0.34		-0.3	-0.42 – -0.19		-240.23	-284.27 – -196.20		-0.00031	--0.0004 – -0.00022		104.14	77.21 – 131.06		11.73	8.72 – 14.74		1.03	-0.44 – 2.50		-0.19	-0.33 – -0.05	
2 alleles	-0.69	-0.85 – -0.53		-0.46	-0.64 – -0.28		-455.95	-521.02 – -390.88		-0.00057	-0.00071 – --0.00044		178.88	137.45 – 220.31		19.81	15.18 – 24.44		0.5	-1.68 – 2.68		-0.31	-0.52 – -0.10	
Interaction term															**0.0008**			**0.001**						
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			279			279		
R^2 / adjusted R^2	0.106 / 0.103			0.058 / 0.055			0.203 / 0.199			0.191/0.189			0.140 / 0.134			0.136 / 0.130			0.086 / 0.073			0.058 / 0.045	

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

	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*
(Intercept)	-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 – 0.01		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)	0.01	-0.02 – 0.04	0.64	0.12	0.06 – 0.18		0.08	0.00 – 0.16	0.09	0.01	-0.02 – 0.05	0.57	0.1	0.05 – 0.15	**0.01**	0.21	0.14 – 0.29	**<0.0001**	0.18	0.07 – 0.28	**0.002**
APOE status (ref = 0 alleles)			0.76						0.34			0.13			0.33			0.27			0.31
1 allele	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	0.02	-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						**0.02**															
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		
R^2 / adjusted R^2	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/Intracranial volume**			**TAU**			**PTAU**			**Cortisol Cortisol ng ml**			**C Reactive Protein ug/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		0.00747	0.00710 – 0.00785		154.16	70.57 – 237.75		15.72	6.36 – 25.09		-1.87	-9.56 – 5.81		-3.19	-3.93 – -2.45	
AGE (years)	-0.01	-0.01 – -0.00		-0.02	-0.03 – -0.01		-1.64	-4.52 – 1.24		-0.00003	-0.00004 – -0.00003		1.25	0.15 – 2.36		0.1	-0.02 – 0.23		0.22	0.12 – 0.32		0.01	-0.00 – 0.02	
Male (ref = Female)	-0.16	-0.23 – -0.09	**<0.0001**	-0.04	-0.13 – 0.05	**<0.0001**	-26.62	-69.46 – 16.22	0.38	-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	0.22	-0.1	-0.24 – 0.03	0.27
Diagnosis (ref = CN)			**<0.0001**			**<0.0001**			**<0.0001**			**<0.0001**			**<0.0001**			**<0.0001**			0.44			0.28
EMCI	-0.5	-0.61 – -0.39		-0.42	-0.56 – -0.27		-85.2	-148.82 – -21.59		-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		-0.00073	-0.00083 – -0.00064		93.34	70.69 – 116.00		10.58	8.05 – 13.12		1.24	-0.42 – 2.90		-0.15	-0.31 – 0.01	
AD	-1.84	-1.94 – -1.75		-1.63	-1.76 – -1.50		-390.48	-453.59 – -327.37		-0.00106	-0.00116 – -0.00096		143.6	119.35 – 167.86		15.81	13.10 – 18.53		0.24	-1.68 – 2.16		-0.15	-0.33 – 0.04	
Observations	1150			1149			947			1234			947			947			279			279		
R^2 / adjusted R^2	0.589 / 0.588			0.380 / 0.377			0.168 / 0.164			0.398 / 0.396			0.164 / 0.160			0.156 / 0.152			0.088 / 0.075			0.030 / 0.016	

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

	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*
(Intercept)	-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 – 0.01		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)	0.01	-0.02 – 0.03	0.77	0.05	0.01 – 0.10	0.06	0.08	0.01 – 0.16	0.11	0.01	-0.02 – 0.05	0.67	0.05	0.01 – 0.09	**0.02**	0.21	0.14 – 0.29	**<0.0001**	0.17	0.06 – 0.27	**0.006**
Diagnosis (ref = CN)			0.18			0.64			0.64			0.64			0.96			0.98			0.67
EMCI																					
LMCI	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	0.03	-0.03 – 0.08		0.03	-0.03 – 0.09		-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		
R^2 / adjusted R^2	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	**0.002**	0.01	-0.01 – 0.04	0.54	-0.15	-0.25 – -0.06	**0.009**	-0.04	-0.06 – -0.01	0.07	0.25	0.15 – 0.34	**<0.0001**	-0.01	-0.04 – 0.03	0.82
APOE status (ref = 0 alleles)			0.29			0.46			**<0.0001**			0.89			0.49			0.41
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.IL 16.pg m**			**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	0.63	0.01	-0.02 – 0.04	0.71	0	-0.06 – 0.05	0.92	-0.03	-0.05 – -0.00	0.19	0.02	-0.02 – 0.06	0.66
APOE status (ref = 0 alleles)			0.66			0.75			0.76			0.54			0.71
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	**0.005**	0.02	-0.01 – 0.04	0.47	-0.14	-0.24 – -0.05	**0.03**	-0.03	-0.06 – -0.01	0.08	0.25	0.15 – 0.34	**<0.0001**	0	-0.04 – 0.03	0.88
Diagnosis (ref = CN)			0.63			**0.01**			0.08			0.27			0.88			0.35
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	0.68	0.01	-0.02 – 0.04	0.63	-0.01	-0.06 – 0.05	0.52	-0.02	-0.05 – -0.00	0.19	0.02	-0.02 – 0.06	0.68
Diagnosis (ref = CN)			0.08			0.08			0.75			0.63			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		