Published Ahead of Print on January 25, 2023 as 10.1212/WNL.0000000000206833

Neurology®
The most widely read and highly cited peer-reviewed neurology journal
The Official Journal of the American Academy of Neurology

Neurology Publish Ahead of Print

DOI: 10.1212/WNL.0000000000206833

Estrogen Receptor Genes, Cognitive Decline, and Alzheimer Disease

Author(s):

Shahram Oveisgharan, MD[1, 2]; Jingyun Yang, PhD[1, 2]; Lei Yu, PhD[1, 2]; Dominika Burba, MS[1]; Woojeong Bang, MS[1]; Shinya Tasaki, PhD[1, 2]; Fran Grodstein, PhD[1, 3]; Yanling Wang, PhD[1]; Jinying Zhao, PhD[4]; Philip Lawrence De Jager, PhD[5, 6]; Julie A. Schneider, MD[1, 2, 7]; David A. Bennett, MD[1, 2]

ACCEPTED

Neurology® Published Ahead of Print articles have been peer reviewed and accepted for publication. This manuscript will be published in its final form after copyediting, page composition, and review of proofs. Errors that could affect the content may be corrected during these processes.

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

Corresponding Author:

Shahram Oveisgharan, shogh2@yahoo.com

Affiliation Information for All Authors: 1. Rush Alzheimer's Disease Center, Rush University Medical Center, Chicago, IL, USA; 2. Department of Neurological Sciences, Rush University Medical Center, Chicago, IL, USA; 3. Department of Internal Medicine, Rush University Medical Center, Chicago, IL, USA; 4. Department of Epidemiology, University of Florida, Gainesville, FL, USA; 5. Center for Translational & Computational Neuroimmunology, Department of Neurology, Columbia University Irving Medical Center, New York, NY, USA; 6. Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University Irving Medical Center, New York, NY, USA; 7. Department of Pathology, Rush University Medical Center, Chicago, IL, USA.

Equal Author Contribution:

Contributions:

Shahram Oveisgharan: Drafting/revision of the manuscript for content, including medical

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

writing for content; Study concept or design; Analysis or interpretation of data

Jingyun Yang: Drafting/revision of the manuscript for content, including medical writing for content; Analysis or interpretation of data

Lei Yu: Drafting/revision of the manuscript for content, including medical writing for content; Analysis or interpretation of data

Dominika Burba: Analysis or interpretation of data

Woojeong Bang: Analysis or interpretation of data

Shinya Tasaki: Drafting/revision of the manuscript for content, including medical writing for content; Analysis or interpretation of data

Fran Grodstein: Drafting/revision of the manuscript for content, including medical writing for content; Analysis or interpretation of data

Yanling Wang: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Jinying Zhao: Drafting/revision of the manuscript for content, including medical writing for content

Philip Lawrence De Jager: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Julie A. Schneider: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

David A. Bennett: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

Figure Count:

5

Table Count:

2

Search Terms:

[26] Alzheimer's disease, [54] Cohort studies, [345] Equity, Diversity, and Inclusion (EDI), [304] Gene expression studies

Acknowledgment:

We thank the study participants and the staff of the Rush Alzheimer's Disease Center.

Study Funding:

The study was supported by NIA grant P30AG10161, P30AG72975, R01AG15819, R01AG17917, U01AG46152, U01AG61356, R01AG52476, and R01AG64786.

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

Disclosures:

The authors report no disclosures relevant to the manuscript.

Preprint DOI:

Received Date:

2022-04-12

Accepted Date:

2022-12-05

Handling Editor Statement:

Submitted and externally peer reviewed. The handling editor was Associate Editor Linda Hershey, MD, PhD, FAAN.

ACCEPTED

ABSTRACT

Background: Lifetime risk of Alzheimer's disease (AD) dementia is two-fold higher in women compared with men, and low estrogen levels in post menopause have been suggested as a possible contributor. We examined 3 *ER*s (*GPER1*, *ER2*, *ER1*) variants in association with AD traits as an indirect method to test the association between estrogen and AD in women. While the

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

study focus was on women, in a comparison we separately examined *ER* molecular variants in men.

Methods: Participants were followed for an average 10 years in one of two longitudinal clinical pathological studies of aging. Global cognition was assessed using a composite score derived from 19 neuropsychological tests' scores. Postmortem pathological assessment included examination of 3 AD (amyloid-β and tau tangles determined by immunohistochemistry, and a global AD pathology score derived from diffuse and neurotic plaques and neurofibrillary tangles count) and 8 non-AD pathology indices. *ER* molecular genomic variants included genotyping and examining *ER* DNA methylation and RNA expression in brain regions including dorsolateral prefrontal cortex (DLPFC) that are major players in cognition and often have AD pathology.

Results: Mean age of women (N=1711) at baseline was 78.0 (SD=7.7) years. In women, *GPER1* molecular variants had the most consistent associations with AD traits. *GPER1* DNA methylation was associated with cognitive decline, tau tangles density, and global AD pathology score. *GPER1* RNA expression in DLPFC was related to cognitive decline and tau tangles density. Other associations included associations of *ER2* and *ER1* SNPs and DNA methylation with cognition. RNA expressions in DLPFC of genes involved in signaling mechanisms of activated ERs were also associated with cognitive decline and tau tangles density in women. In men (N=651, average age at baseline: 77.4 (SD=7.3)), there were less robust associations between *ER* molecular genomic variants and AD cognitive and pathological traits. No consistent association was seen between *ER* molecular genomic variations and non-AD pathologies in either of sexes.

Conclusion: *ER* DNA methylation and RNA expression, and to some extent *ER* polymorphisms, were associated with AD cognitive and pathologic traits in women, and to a lower extent in men.

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

INTRODUCTION

Women comprise more than 60% of Americans with Alzheimer's dementia[1], and lifetime risk of Alzheimer's dementia is two-fold higher in women compared with men[2]. As older women lose their main source of estrogen after menopause, low estrogen levels[3] have been proposed a possible contributor for vulnerability of older women for Alzheimer's dementia[4]. Prior studies suggested that older age at menopause and longer reproductive age were associated with higher levels of cognition in older women[5,6]. Low bioavailable levels of estradiol in older women were associated with a higher risk of cognitive impairment[7]. However, results of randomized clinical trials that examined postmenopausal hormone therapy for prevention of cognitive decline have found no benefits[8,9]. These inconsistent findings could stem from different reasons including locally generated estrogen that affects brain function[10] above and beyond circulating estrogen. Therefore, other avenues are needed to explore to clarify whether estrogen is related to cognition in older women.

Estrogen has 1 transmembrane receptor, G protein-coupled estrogen receptor (GPER1), and 2 steroid receptors, estrogen receptor α (ER1) and β (ER2), that are the main receptors mediating estrogen activity. Because of the possible association between estrogen and cognition[5,7], several cross-sectional[11,12] and few longitudinal studies[13–15] have examined *ER1* and *ER2* polymorphisms with cognitive impairment or cognitive decline. In these studies, only a few selected polymorphisms were examined. In the current study, the overarching research question was to examine associations between *ER* molecular genomic variants with cognition in older women, and we extended prior studies in 4 areas. First, we examined common single nucleotide polymorphisms (SNPs) in and flanking *ER1* and *ER2*. Second, we also examined *GPER1* polymorphisms whose SNPs were associated with hormone-sensitive diseases[16,17] but were not

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

examined in relation to cognition. Third, we examined *ER* SNPs with Alzheimer's disease and related dementias pathologic indices. Fourth, we examined DNA methylation and RNA expression of the *ER* genes with cognitive decline, and with the pathologic indices. While the study focus was on women, in a comparison we separately examined men *ER* molecular variants.

METHODS

Participants were enrolled in one of two longitudinal studies of community-dwelling older adults: The Religious Orders Study (ROS) and the Rush Memory and Aging Project (MAP). One of the authors, David A Bennett, is the principle investigator of both studies. ROS began enrollment in 1994 and enrolls nuns, priests, and brothers across the US. MAP began enrollment in 1997 and enrolls older adults living in personal accommodations or retirement centers across Northeastern Illinois. Eligible participants were older adults without known dementia at enrollment who consented to annual cognitive and clinical assessment and for brain donation at the time of death. Implementing the same protocols and performing the cognitive and clinical assessments by the same trained personnel facilitated joint analysis of ROS and MAP whose details are described elsewhere[18].

We included 1711 women without dementia at baseline, with available genotype data, and with at least two cognitive assessments. Of the 1711 women, 917 died and had available postmortem pathological examinations. Postmortem *ER* methylation data were available in 420 and *ER* expression levels in 738. Corresponding numbers of men were 651 with genotype data, 222 with methylation data, and 323 with RNA expression data. Flow charts of the analytic samples are illustrated in **eFigures 1–2 in the Supplement**. Venn diagrams illustrate sample sizes across different datasets (**eFigures 3–4**).

Genotype processing

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

ACCEPTED

DNA was extracted from peripheral blood or frozen brain tissue. Genotyping was performed on the Affymetrix Genome-Wide Human SNP Array6.0 or the Illumina Omni Quad Express platform. After passing quality control steps[19], SNP dosages were imputed on the Haplotype Reference Consortium (HRC) panel. In this study, we examined SNPs located in the 3 *ER* genic regions and their 10-kilobase flanking areas. SNPs were excluded if they had minor allele frequency < 1%, imputation score < 0.3, or missing rate > 5%. Because the study's primary objective was at the gene level, rather than SNP level, we did not prune SNPs based on their linkage disequilibrium. This yielded 141 SNPs in *GPER1*, 697 in *ER2*, and 1547 in *ER1* regions to be examined in this study.

DNA Methylation processing

Frozen samples of dorsolateral prefrontal cortex (DLPFC) were used to obtain DNA methylation data. DLPFC was selected as the brain region for molecular assessments because it is crucially involved in cognitive activities and frequently has AD pathology in older adults.

DNA was extracted from DLPFC gray mater using Qiagen QIAamp DNA mini protocol. DNA methylation was generated using a bead assay (Infinium HumanMethylation450; Illumina). Raw methylation data were processed further for quality control[20], which resulted in distinct DNA methylation values for 420132 autosomal CpG sites. In this study, we examined CpG sites located in the 3 *ER* genic regions and their 10-kilobase flanking areas. This yielded 84 CpG sites in *GPER1*, 50 in *ER2*, and 76 in *ER1* regions to be examined in this study.

RNA Expression processing

DLPFC was the first brain region at which RNA extraction and processing was performed[21]. RNAs were extracted from frozen samples of DLPFC using Qiagen's miRNeasey mini kit and

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

ACCEPTED

the RNase free Dnase Set. RNA samples that passed quality control criteria were sequenced on Illumina HiSeq platform after RNA-seq library preparation using the strand-specific dUTP method with poly(A) selection. RNA-seq data were processed further keeping only highly expressed genes, which resulted in 13484 genes.

Recently, we generated TruSeq stranded Total RNA Library libraries (Illumina, 20020599) from DLPFC, posterior cingulate cortex (PCC), and anterior cingulate cortex (ACC) using a Zephyr G3 NGS workstation (Perkin Elmer) according to the manufacturer's instructions with minor modifications[22]. Libraries was sequenced on an Illumina NovaSeq 6000 platform at 40-50M reads, 2x150bp paired-end. While expression levels of *GPER1* and *ER2* were available, *ER1* expression level did not meet quality control criteria.

Cognition assessment

Annually, 21 neuropsychological tests (**eMethods in the Supplement**) were administered by research assistants who worked in both cohorts and were trained by a single trainer. Two tests were used only for diagnostic purposes, and scores of the remaining 19 tests, which were the same tests across ROSMAP participants and across visits, were standardized using means and standard deviations of all participants' scores at baseline. Global cognition score is the mean of the 19 standardized neuropsychological scores. We have used these composite measures of cognition in our prior studies[23,24] because composite variables derived from multiple indicators are more appropriate for use in longitudinal analysis[25] where we need variables with less random errors including floor and ceiling effects.

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

At each annual visit, a neurologist and a neuropsychologist reviewed cognitive and clinical data and adjudicated presence of mild cognitive impairment (MCI) or dementia based on established criteria[26].

Postmortem pathological assessment

The median (Q1-Q3, range) of time interval between death and autopsy was 6.8 (5.1–10.2, 1–76.3) hours. Details of the procedures are described elsewhere[18,27]. One hemisphere was frozen for multi omics and biochemical analyses, and the next hemisphere was fixed in 4% formaldehyde solution. The fixed hemisphere was cut into 1 cm slabs, and tissue sections were prepared from pre-determined brain regions that were examined for Alzheimer's disease (AD) and non-AD pathologies by experts blinded to clinical data.

AD. Sections from 8 brain regions were examined by immunohistochemistry for assessment of amyloid-β (Aβ) and tau-labeled tangles using antibodies against Aβ and phosphorylated tau[28]. Using image analysis, compartments labeled by Aβ antibodies were quantified in each brain region as the percent area occupied by the labeled compartment, which was averaged across brain regions to yield the overall Aβ load. Similarly, the overall tau tangle density of brain was calculated by averaging regional tau labeled tangles, which were counted using stereological mapping techniques.

A modified Bielschowsky silver stain was used to visualize neuritic plaques, diffuse plaques, and neurofibrillary tangles in 5 cortical brain regions. A global AD pathologic score was developed by making an average of the summary measures of the 3 indices. Moreover, a board-certified neuropathologist blinded to clinical data determined pathological AD diagnosis using established criteria[29].

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

Non-AD pathologies. We also examined indices of 3 neurodegenerative [Lewy bodies, TDP-43, hippocampal sclerosis] and 5 cerebrovascular disease [macroinfarcts, microinfarcts, atherosclerosis, arteriolosclerosis, cerebral amyloid angiopathy (CAA)] pathologies, which are described in the **eMethods** and in previous studies[27].

Covariates

Sex, race, and years of education were obtained using self-report at baseline. Age at baseline and age at death were calculated using dates of birth (obtained at baseline), baseline interview, and death.

Statistical analysis

Characteristics of participants in different analytic datasets were compared using analysis of variance, chi-square, and Kruskal-Wallis tests. Linear mixed effects models were used to examine cognitive decline over years of follow up. The core model had terms for intercept (level of cognition), time (rate of cognitive decline), age, education, and interactions of age and education with time. Time was treated differently in models examining SNPs compared with models examining DNA methylation or RNA expression. In models with SNPs, the time term of the core mixed effects model was analyzed prospectively. Time was zero at study baseline and was positive thereafter over years of follow up. By contrast, levels of DNA methylation and RNA expression were determined at death and time was zero at death and negative in years prior to death.

Then, in separate mixed effects models we examined the association of each SNP's dosage with the level and rate of cognitive decline by addition of terms for the SNP and its interaction with time, respectively. P values of the associations of SNPs in each *ER* genic region were combined

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

ACCEPTED

separately for the level and rate of cognitive decline using the Fisher product method[30], as described previously[31]. This omnibus method tests a null hypothesis that none of the examined SNPs at an *ER* gene were associated with the outcome (level or rate of cognitive decline). The omnibus test can handle arbitrary dependency structures, is powerful and computationally efficient for boosting power in sequencing studies[32]. When an omnibus test indicated association of a gene's polymorphisms with an outcome, we subsequently examined each SNP to identify specific SNPs associated with the outcome. In further analyses, we replaced SNPs with DNA methylation level at each CpG site or with mRNA expression levels of *ER* genes.

Then, we examined associations of *ER* polymorphisms, methylation, and expression levels with AD and non-AD pathologic indices. We replaced mixed effects models with linear regressions, when examining AD pathology indices, or logistic regressions, when examining non-AD pathologies.

Models assumptions were assessed both graphically and numerically. We applied Benjamini and Hochberg false discovery rate (FDR)[33] to reduce inflation of Type-I error due to multiple testing. For parsimony, only FDR-corrected p-values (q-values) are reported, and q-values less than 0.05 were indicators for rejection of null hypotheses.

Standard Protocol Approvals, Registrations, and Patient Consents

All participants signed an informed consent and an Anatomical Gift Act. Rush University Medical Center Institutional Review Board (IRB) approved each study. The IRB approval numbers are L91020181 (ROS) and L86121802 (MAP).

Data availability

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

The data can be obtained via the Rush Alzheimer's Disease Center Research Resource Sharing Hub at *www.radc.rush.edu*. To access data, an application must be filled including study premise and a brief description of the research plan.

RESULTS

Demographic and other characteristics of the 3 datasets of women used for analyzing SNPs, DNA methylation, and RNA expression are shown in **Table 1**. At baseline, the average age of women in the SNPs dataset was 78 years old, which was 3 years younger than the other 2 datasets that were composed of deceased participants (**eTable 1 in the Supplement**). Although level of education was not different across the 3 datasets, level of cognition at baseline was the highest in the SNPs dataset consistent with being youngest at baseline (**eTable 1**).

Women in the SNPs dataset had the longest follow up, which was 10 years on average. At baseline, 24% met MCI diagnosis and none had dementia while at the last visit, done on average 1 year before death, 24% had MCI and 43% had dementia. Dementia was more frequent in participants with AD pathological diagnosis than without (55% vs. 21%, χ^2=97.5, df=1, p<0.001).

Associations of *ER* polymorphisms, DNA methylation and RNA expression in women are summarized in **Table 2**, and are explicated below.

GPER1-Women

Polymorphisms-cognition. Using mixed effects models and subsequent omnibus tests indicated that *GPER1* polymorphisms were associated with level of global cognition at baseline (q=0.028) but not with the rate of cognitive decline. Examining individual SNPs indicated that 1 (1%) SNP was associated with level of global cognition (**Figure 1; eTable 2 in the Supplement**).

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

Polymorphisms-pathologies. The omnibus tests indicated that *GPER1* polymorphisms were not associated with AD pathologic indices. In examining associations with non-AD pathologies, *GPER1* polymorphisms were associated with only microinfarcts (q=0.038). Examining individual SNPs separately, 11 SNPs were associated with microinfarcts after FDR correction (**eTable 3 in the Supplement**).

DNA methylation-cognition. Rate of cognitive decline (q<0.001) and cognition level at death (q<0.001) were both related to levels of *GPER1* DNA methylation measured postmortem. Examining individual CpG sites indicated that most of significant CpG sites were related to both rate of cognitive decline and cognition level (**Figure 1; eTables 4–6 in the Supplement**).

DNA methylation-pathologies. DNA methylation at *GPER1* was associated with tau tangles density (q=0.013) and global AD pathology score (q=0.013). Examining individual CpG sites indicated that DNA methylation values of 10 and 14 CpG sites at *GPER1* were associated with tau tangle density and global AD pathology score, respectively, after FDR correction (**Figure 2, eTable 7**).

DNA methylation at *GPER1* was not associated with any of the non-AD pathologies.

RNA expression-cognition. Both rate of cognitive decline (Estimate=-0.014, SE=0.004, q=0.005) and final level of cognition at death (Estimate=-0.181, SE=0.045, q<0.001) were related to *GPER1* expression levels in postmortem DLPFC (**Figure 3**). To contextualize the effect size, we used model-derived estimates (**eTable 8**) to compare the rate of cognitive decline in two representative women with different levels of DLPFC *GPER1* RNA expression. Cognitive decline in a woman with high (90th percentile=14.9) level of *GPER1* RNA expression was 35% faster than a woman with low (10th percentile=12.8) level.

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

ACCEPTED

Only level of cognition (Estimate=-0.201, SE=0.076, q=0.026), not rate of decline (Estimate=-0.015, SE=0.007, q=0.083), was related to expression levels of *GPER1* at PCC (**Figure 3**). *GPER1* expression levels at ACC were not associated with cognitive decline.

RNA expression-pathologies. Examining associations of *GPER1* expression levels with AD pathologic indices indicated that level of expression in DLPFC was associated with tau tangles density (Estimate=0.140, SE=0.051, q=0.039) but not Aβ load or global AD pathology score (**Figure 4**). To contextualize the effect size, we used model-derived estimates (**eTable 9 in the Supplement**) to compare tau tangles density in two representative women with different levels of DLPFC *GPER1* RNA expression. Tau tangles density was 19% higher in a woman with high (90th percentile) level of *GPER1* RNA expression compared with low (10th percentile) level. No association was seen between expression levels of *GPER1* at PCC or ACC and AD pathologic indices. In examining non-AD pathologies, *GPER1* expression was not associated with any of them.

DNA methylation-RNA expression. Because both DNA methylation and RNA expression of GPER1 in DLPFC were related to cognitive decline and tau tangles density, we examined their association by themselves. DNA methylation and RNA expression of *GPER1* in DLPFC were not related.

ER2-Women

Polymorphisms-cognition. ER2 polymorphisms were associated with level of global cognition at baseline (q=0.009) but not rate of cognitive decline. Examining individual SNPs indicated that 42 (6%) of SNPs were associated with the baseline level of global cognition (**Figure 1; eTable 2 in the Supplement**).

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

Polymorphisms-pathologies. ER2 polymorphisms were neither associated with AD nor with non-AD pathologies.

DNA methylation-cognition. Like *GPER1* DNA methylation, rate of cognitive decline (q<0.001) and cognition level at death (q<0.001) were related to levels of *ER2* DNA methylation measured postmortem. Examining individual CpG sites indicated that all 5 significant CpG sites were related to both rate of cognitive decline and cognition level (**Figure 1; eTables 4-6 in the Supplement**).

DNA methylation-pathologies. DNA methylation at *ER2* CpG sites was associated with tau tangle density (q=0.013), but not with Aβ load or global AD pathology score. By examining individual CpG sites, we found that DNA methylation value of 1 CpG site at *ER2* was associated with tau tangle density (**Figure 2, eTable 7 in the Supplement**).

DNA methylation at *ER2* was not associated with any of the non-AD pathologies.

RNA expression-cognition. In mixed effects models, *ER2* expression levels were not associated with cognitive decline.

RNA expression-pathologies. Expression levels of *ER2* at DLPFC and PCC were associated with only global AD pathology score (DLPFC: Estimate=0.048, SE=0.019, q=0.035; PCC: Estimate=0.074, SE=0.029, q=0.035) not Aβ or tau tangles (**Figure 5**). Expression level of *ER2* at ACC was not associated with any of the AD pathology indices.

Examining non-AD pathologies, *ER2* expression level was not associated with any of them.

ER1-Women

Polymorphisms-cognition. ER1 polymorphisms were associated with level of global cognition at baseline (q<0.001) but not rate of cognitive decline. Examining individual SNPs indicated that 82 (5%) SNPs were associated with level of global cognition (**Figure 1; eTable 2**).

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

Polymorphisms-pathologies. ER1 polymorphisms were neither associated with AD nor with non-AD pathologies.

DNA methylation-cognition. Like *GPER1* and *ER2* DNA methylation, rate of cognitive decline (q<0.001) and cognition level at death (q<0.001) were related to levels of *ER1* DNA methylation measured postmortem. Examining individual CpG sites indicated that more than half of significant CpG sites were related to both rate of cognitive decline and cognition level (**Figure 1; eTables 4–6 in the Supplement**).

DNA methylation-pathologies. Gene-based analysis indicated that DNA methylation at *ER1* was associated with tau tangle density (q=0.016), but not with Aβ load or global AD pathology score. Examining individual CpG sites indicated that DNA methylation value of 1 CpG site at *ER1* was associated with tau tangle density (**Figure 2, eTable 7 in the Supplement**).

DNA methylation at *ER1* was not associated with any of the examined non-AD pathologies.

Sensitivity analyses

Because *GPER1* RNA expression in DLPFC had the most consistent association with AD phenotypes in women, we did four sensitivity analyses to confirm this finding. First, we separately examined *GPER1* RNA expression in DLPFC with cognitive decline in women with and without AD pathological diagnoses. The findings confirmed our hypothesis that the associations of *GPER1* RNA expression with cognitive decline was stronger in women with an AD pathological diagnosis (**eTable 10 in the Supplement**). Second, we hypothesized that if *GPER1* RNA expression was associated with AD traits should also be RNA expressions of genes of signaling pathways of activated ERs. We examined DLPFC RNA expressions of three signaling pathways of activated ERs: c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase, and Akt kinase[34]. We found that the most robust associations with cognitive

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

ACCEPTED

decline and tau tangles density were derived from analyzing RNA expression of genes of JNK pathway, which are more specifically involved in downstream signaling of activated GPER1 rather than ER1 and ER2[34] (**eTables 11–12**). Third, we removed the first three global cognition assessments to address a learning effect that occurs during the first annual visits[35], as done previously[36]. However, removal of the first three cognition assessments did not change our findings that rate of cognitive decline (Estimate=-0.012, SE=0.005, p=0.030) and final level of cognition at death (Estimate=-0.012, SE=0.005, p=0.030) were related to *GPER1* expression levels in postmortem. Fourth, we randomly selected 100 genes whose RNA expression levels in DLPFC passed quality-control criteria. Then, in separate models we examined associations of the 100 RNA expressions with cognitive decline and AD pathology indices (**eTables 13–14**). The analyses indicated that 4 genes had the same type of associations that *GPER1* had: their RNA expressions were associated with faster cognitive decline and more tau tangles. The 4 genes were *ZC3H11A*, *FURIN*, *SMARCD3*, and *PPP4R2*, and 2 of them were previously reported associated with gray matter volume (*ZC3H11A*)[37] and AD (*FURIN*)[38]. These sensitivity analyses supported the association of *GPER1* RNA expression in DLPFC with AD phenotypes in women

ER-Men

As a comparison, we examined associations of the *ER* molecular variants with cognitive decline and the pathology indices in men (**eTable 15 in the Supplement**) whose characteristics are shown in **Table 1**. *ER1* SNPs were the only *ER* SNPs associated with cognition (**eTable 16**). Moreover, *ER1* DNA methylation sites were the only ones associated with cognition and AD pathology indices (**eTables 17–18**). RNA expressions of *GPER1* and *ER2* were not associated with cognition or AD pathology indices in men. None of molecular variants of *ER* genes were

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

associated with non-AD pathologies. These findings suggest that in men there were less robust associations between *ER* molecular genomic variants and AD traits.

DISCUSSION

In this study, *ER* polymorphisms were not associated with rate of cognitive decline in women, which was supported by lack of association between *ER* polymorphisms and AD pathologic indices and is in line with meta-analyses indicating lack of association between *ER* polymorphisms and dementia in European ancestry[39,40]. Although few longitudinal studies reported association between some *ER1* and *ER2* polymorphisms and cognitive decline[14,15], their samples were younger than ours and were followed for fewer years. To our knowledge, this is the first study that examined *ER* polymorphisms with AD and non-AD brain pathologies or examined *ER* DNA methylation and expression levels with cognitive decline and brain pathologies.

In contrast to polymorphisms, DNA methylation at all 3 *ER* genes in women was associated with rate of cognitive decline and level of tau tangle density, which is the major AD pathologic index that drives cognitive decline[41]. Moreover, *GPER1* expression levels in women were associated with rate of cognitive decline and with some indices of AD pathology. These results are consistent with previous studies in women in which ER mediates effects of estrogen and DNA methylation is necessary for estrogen to enhance memory consolidation[34]. In fact, our finding that levels of DNA methylation and RNA expression of *GPER1* in DLPFC were not related while both were associated with cognitive decline and tau tangles density indicates that the associations of *ER* DNA methylation with AD traits were not through alteration of *ER* expression but were rather through other mechanisms. Several mechanisms have been attributed to estrogen's possible effect on cognition, that may also underlie the association of cognition with

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

GPER1 RNA expression as a mediator of estrogen effects, including modulation of neurotransmitters (acetylcholine[42] and dopamine[43]), enhancement of memory consolidation pathways (long term potentiation and depression and dendritic spine density[44]), and neuroprotective mechanisms[45]. However, few studies have examined whether estrogens or ER are associated with production or clearance of Aβ load or tau tangles density[46,47]. Although randomized trials examining estrogen for prevention of cognitive decline in women[8,9] were negative, several factors may underlie these negative findings including type of menopause of studied women, women's age at enrollment, type of treatment, its dosage, or its mode of delivery. More studies are required to uncover whether the current findings are reproducible and valid.

Compared with *ER1* and *ER2*, *GPER1* DNA methylation and RNA expression had the most consistent associations with AD pathologic indices, cognition level, and cognitive decline rate in older women. While more consistent associations of *GPER1* may be due to its specific downstream signaling mechanisms including JNK pathway[34], it may also be due to the brain regions examined in this study. Prior studies indicated that GPER1 had the highest level in prefrontal cortex compared with ER1 and ER2[43]. ER1 was located primarily in amygdala and hypothalamus[48] and higher levels of ER2 were present in hippocampus compared with ER1[43,48]. Therefore, brain regions with the highest levels of ER1 and ER2 were not examined in this study, which might have favored preferential associations of *GPER1* with AD traits in women.

While the study focus was more on women, we also separately examined men *ER*. In men, there were less robust associations between *ER* molecular genomic variants and AD traits. Moreover, they were *ER1*, rather than *GPER1* and *ER2*, SNPs and DNA methylation that had associations with cognitive decline and AD pathology indices. This finding is corroborated by a meta-analysis

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

suggesting association of an *ER1* SNP with cognition in men not women[11]. Future studies are needed to clarify whether this heterogenous associations of *ER* molecular variants with AD cognitive and pathological phenotypes across sexes is related to higher levels of estrogen in older men compared with postmenopausal women.

We did not find a consistent association between the 3 *ER* genes' polymorphisms, DNA methylation, or RNA expression and non-AD brain pathologies including vascular pathologies in women or men. To our knowledge, no prior studies examined these associations. Prior studies examining *ER* genes in relation to peripheral vessels' atherosclerosis or clinical vascular events did not report consistent associations either[49,50]. Therefore, further studies are required to untangle whether no association exists between *ER* genes and non-AD brain pathologies, or some associations will be found by using more granular metrics for non-AD pathological assessments.

Clinicians confront conflicting studies about potential benefits of estrogen on cognition. This study suggests that there is substantial heterogeneity among *ER* molecular genomics in relation to AD cognitive and pathological traits in older women, which may underlie the conflicting relationship between estrogen and cognition. We examined *ER* molecular genomics in DLPFC that strongly contributes to cognition. Higher expressions of *GPER1*, not *ER2*, were related to faster cognitive decline. Moreover, higher levels of all 3 *ER* DNA methylation were related to cognitive decline through unidentified mechanisms. Eventually, translating this kind of work to living humans will be required to make our findings actionable for clinicians.

Some study strengths underlie our findings. Data came from well-characterized longitudinal studies of older adults with high follow up and autopsy rates. Staff involved in the assessment of brain pathologies or in the measurement of genetic variations were blinded to clinical data.

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

However, some limitations require further studies to confirm our findings. The participants were selected, not representative of the US population. They were mostly non-Hispanic white volunteers with high education level, which requires findings to be replicated in more diverse populations, including more participants of minorities and more participants with low socioeconomic status, for generalizability. The cohorts did not include questions to differentiate cisgender from transgender women or men. The postmortem interval was not short, which might have affected *ER* DNA methylation and RNA expression levels. Not everyone with genetic information had available DNA methylation or transcriptomic data. Due to postmortem assessment of DNA methylation or RNA expression, a temporal order of their occurrence in relation to the examined outcomes, cognitive decline or AD pathologies, cannot be inferred. Our DNA methylation data were produced using Illumina 450K array that targets regions with a high frequency of CpG sites, which is a small fraction of the whole epigenome. Although we developed a composite measure of global cognition from 19 neuropsychological tests, the measure might still have suffered from distributional properties, including floor and ceiling effects, of the included tests.

In conclusion, we found that *ER* molecular variants were associated with AD phenotypes in women. *ER* polymorphisms were associated with baseline level of cognition. *ER* genes methylation and expression levels, specifically *GPER1* in DLPFC, were associated with rate of cognitive decline, level of cognition at death, and AD pathology indices. In men, there were less robust associations between *ER* molecular genomic variants and AD traits. Nonetheless, no consistent association was seen between *ER* and non-AD pathologies in either of sexes. Further studies are required to confirm the above findings.

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

Appendix 1: Authors.

Name	**Location**	**Contribution**
Shahram Oveisgharan, MD	Rush University Medical Center	Design and conceptualized study; Interpretation of the data; Drafted the manuscript for intellectual content
Jingyun Yang, PhD;	Rush University Medical Center	Analysis and interpretation of data; revising the manuscript for intellectual content
Lei Yu, PhD	Rush University Medical Center	Analysis and interpretation of data; revising the manuscript for intellectual content
Dominika Burba, MS	Rush University Medical Center	Analysis of data;
Woojeong Bang, MS	Rush University Medical Center	Analysis of data;
Shinya Tasaki, PhD	Rush University Medical Center	Analysis and interpretation of data; revising the manuscript for intellectual content
Fran Grodstein, PhD	Rush University Medical Center	Interpretation of data; revising the manuscript for intellectual content

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

Yanling Wang, PhD	Rush University Medical Center	Acquisition of data; revising the manuscript for intellectual content
Jinying Zhao, PhD	University of Florida	Revising the manuscript for intellectual content
Philip L De Jager, MD, PhD	Columbia University Irving Medical Center	Acquisition of data; revising the manuscript for intellectual content
Julie A Schneider, MD	Rush University Medical Center	Acquisition of data; revising the manuscript for intellectual content
David A. Bennett, MD	Rush University Medical Center	Acquisition of data; Design and conceptualized study; Interpretation of the data; revising the manuscript for intellectual content

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

WNL-2022-201632_sup --- http://links.lww.com/WNL/C615

REFERENCES

1. 2021 Alzheimer's disease facts and figures. Alzheimers Dement. 2021;17:327–406.

2. Chêne G, Beiser A, Au R, et al. Gender and incidence of dementia in the Framingham Heart Study from mid-adult life. Alzheimers Dement. 2015;11:310–320.

3. Davis SR, Martinez-Garcia A, Robinson PJ, et al. Estrone Is a Strong Predictor of Circulating Estradiol in Women Age 70 Years and Older. J Clin Endocrinol Metab. 2020;105:dgaa429.

4. Yaffe K, Sawaya G, Lieberburg I, Grady D. Estrogen therapy in postmenopausal women: effects on cognitive function and dementia. JAMA. 1998;279:688–695.

5. Kuh D, Cooper R, Moore A, Richards M, Hardy R. Age at menopause and lifetime cognition: Findings from a British birth cohort study. Neurology. 2018;90:e1673–e1681.

6. Georgakis MK, Kalogirou EI, Diamantaras A-A, et al. Age at menopause and duration of reproductive period in association with dementia and cognitive function: A systematic review and meta-analysis. Psychoneuroendocrinology. 2016;73:224–243.

7. Yaffe K, Lui LY, Grady D, Cauley J, Kramer J, Cummings SR. Cognitive decline in women in relation to non-protein-bound oestradiol concentrations. Lancet. 2000;356:708–712.

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

8. O'Brien J, Jackson JW, Grodstein F, Blacker D, Weuve J. Postmenopausal hormone therapy is not associated with risk of all-cause dementia and Alzheimer's disease. Epidemiol Rev. 2014;36:83–103.

9. Zhou H-H, Yu Z, Luo L, Xie F, Wang Y, Wan Z. The effect of hormone replacement therapy on cognitive function in healthy postmenopausal women: a meta-analysis of 23 randomized controlled trials. Psychogeriatrics. 2021;21:926–938.

10. Brann DW, Lu Y, Wang J, et al. Brain-derived estrogen and neural function. Neurosci Biobehav Rev. 2022;132:793–817.

11. Lee YH, Song GG. Estrogen receptor 1 PvuII and XbaI polymorphisms and susceptibility to Alzheimer's disease: a meta-analysis. Genet Mol Res. 2015;14:9361–9369.

12. Ulhaq ZS, Garcia CP. Estrogen receptor beta (ESR2) gene polymorphism and susceptibility to dementia. Acta Neurol Belg. 2021;121:1281–1293.

13. Yaffe K, Lui LY, Grady D, Stone K, Morin P. Estrogen receptor 1 polymorphisms and risk of cognitive impairment in older women. Biol Psychiatry. 2002;51:677–682.

14. Yaffe K, Lindquist K, Sen S, et al. Estrogen receptor genotype and risk of cognitive impairment in elders: findings from the Health ABC study. Neurobiol Aging. 2009;30:607–614.

15. Ryan J, Carrière I, Amieva H, et al. Prospective analysis of the association between estrogen receptor gene variants and the risk of cognitive decline in elderly women. Eur Neuropsychopharmacol. 2013;23:1763–1768.

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

16. Giess M, Lattrich C, Springwald A, Goerse R, Ortmann O, Treeck O. GPR30 gene polymorphisms are associated with progesterone receptor status and histopathological characteristics of breast cancer patients. J Steroid Biochem Mol Biol. 2010;118:7–12.

17. Chevalier N, Paul-Bellon R, Camparo P, Michiels J-F, Chevallier D, Fénichel P. Genetic variants of GPER/GPR30, a novel estrogen-related G protein receptor, are associated with human seminoma. Int J Mol Sci. 2014;15:1574–1589.

18. Bennett DA, Buchman AS, Boyle PA, Barnes LL, Wilson RS, Schneider JA. Religious Orders Study and Rush Memory and Aging Project. J Alzheimers Dis. 2018;64:S161–S189.

19. Shulman JM, Chen K, Keenan BT, et al. Genetic susceptibility for Alzheimer disease neuritic plaque pathology. JAMA Neurol. 2013;70:1150–1157.

20. De Jager PL, Srivastava G, Lunnon K, et al. Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci. Nat Neurosci. 2014;17:1156–1163.

21. Mostafavi S, Gaiteri C, Sullivan SE, et al. A molecular network of the aging human brain provides insights into the pathology and cognitive decline of Alzheimer's disease. Nature neuroscience. 2018;21:811–819.

22. Tasaki S, Xu J, Avey DR, et al. Inferring protein expression changes from mRNA in Alzheimer's dementia using deep neural networks. Nat Commun. 2022;13:655.

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

23. Oveisgharan S, Buchman AS, Yu L, et al. APOE epsilon2epsilon4 genotype, incident AD and MCI, cognitive decline, and AD pathology in older adults. Neurology. 2018;90:e2127–e2134.

24. Oveisgharan S, Wilson RS, Yu L, Schneider JA, Bennett DA. Association of Early-Life Cognitive Enrichment With Alzheimer Disease Pathological Changes and Cognitive Decline. JAMA Neurol. 2020;77:1217.

25. von Oertzen T, Hertzog C, Lindenberger U, Ghisletta P. The effect of multiple indicators on the power to detect inter-individual differences in change. Br J Math Stat Psychol. 2010;63:627–646.

26. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2011;7:263–269.

27. Oveisgharan S, Arvanitakis Z, Yu L, Farfel J, Schneider JA, Bennett DA. Sex differences in Alzheimer's disease and common neuropathologies of aging. Acta Neuropathol. 2018;136:887–900.

28. Oveisgharan S, Capuano AW, Kapasi A, et al. Association of low systolic blood pressure with postmortem amyloid-β and tau. J Alzheimers Dis. 2020;78:1755–1764.

29. Hyman BT, Trojanowski JQ. Editorial on consensus recommendations for the postmortem diagnosis of Alzheimer disease from the National Institute on Aging and the Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

Alzheimer disease: Journal of Neuropathology and Experimental Neurology. 1997;56:1095–1097.

30. Fisher R. Questions and answers #14. Am Stat. 1948;2:30–31.

31. Yu L, Chibnik LB, Srivastava GP, et al. Association of Brain DNA methylation in SORL1, ABCA7, HLA-DRB5, SLC24A4, and BIN1 with pathological diagnosis of Alzheimer disease. JAMA Neurol. 2015;72:15–24.

32. Liu Y, Xie J. Cauchy combination test: a powerful test with analytic p-value calculation under arbitrary dependency structures. J Am Stat Assoc. 2020;115:393–402.

33. Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B (Methodological). 1995;57:289–300.

34. Frick KM. Molecular mechanisms underlying the memory-enhancing effects of estradiol. Horm Behav. 2015;74:4–18.

35. Wilson RS, Capuano AW, Yu L, et al. Neurodegenerative disease and cognitive retest learning. Neurobiol Aging. 2018;66:122–130.

36. Wilson RS, Wang T, Yu L, Bennett DA, Boyle PA. Normative Cognitive Decline in Old Age. Ann Neurol. 2020;87:816–829.

37. Wei K, Kong W, Wang S. Integration of Imaging Genomics Data for the Study of Alzheimer's Disease Using Joint-Connectivity-Based Sparse Nonnegative Matrix Factorization. J Mol Neurosci. 2022;72:255–272.

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

38. Zhang Y, Gao X, Bai X, Yao S, Chang Y-Z, Gao G. The emerging role of furin in neurodegenerative and neuropsychiatric diseases. Transl Neurodegener. 2022;11:39.

39. Luckhaus C, Sand PG. Estrogen Receptor 1 gene (ESR1) variants in Alzheimer's disease. Results of a meta-analysis. Aging Clin Exp Res. 2007;19:165–168.

40. Ulhaq ZS, Garcia CP. Estrogen receptor beta (ESR2) gene polymorphism and susceptibility to dementia. Acta Neurol Belg. 2021;121:1281–1293.

41. Bennett DA, Schneider JA, Wilson RS, Bienias JL, Arnold SE. Neurofibrillary tangles mediate the association of amyloid load with clinical Alzheimer disease and level of cognitive function. Arch Neurol. 2004;61:378–384.

42. Newhouse P, Dumas J. Estrogen-cholinergic interactions: Implications for cognitive aging. Horm Behav. 2015;74:173–185.

43. Almey A, Milner TA, Brake WG. Estrogen receptors in the central nervous system and their implication for dopamine-dependent cognition in females. Horm Behav. 2015;74:125–138.

44. Frankfurt M, Luine V. The evolving role of dendritic spines and memory: Interaction(s) with estradiol. Horm Behav. 2015;74:28–36.

45. Arevalo M-A, Azcoitia I, Garcia-Segura LM. The neuroprotective actions of oestradiol and oestrogen receptors. Nat Rev Neurosci. 2015;16:17–29.

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

46. Xiong Y-S, Liu F-F, Liu D, et al. Opposite effects of two estrogen receptors on tau phosphorylation through disparate effects on the miR-218/PTPA pathway. Aging Cell. 2015;14:867–877.

47. Hwang CJ, Yun H-M, Park K-R, et al. Memory Impairment in Estrogen Receptor α Knockout Mice Through Accumulation of Amyloid-β Peptides. Mol Neurobiol. 2015;52:176–186.

48. Sundermann EE, Maki PM, Bishop JR. A review of estrogen receptor alpha gene (ESR1) polymorphisms, mood, and cognition. Menopause. 2010;17:874–886.

49. Kjaergaard AD, Ellervik C, Tybjaerg-Hansen A, et al. Estrogen receptor alpha polymorphism and risk of cardiovascular disease, cancer, and hip fracture: cross-sectional, cohort, and case-control studies and a meta-analysis. Circulation. 2007;115:861–871.

50. Li B-H, Zhang L-L, Yin Y-W, et al. Association between estrogen receptor alpha c.454-397T>C and c.454-351A>G and ischemic stroke risk: a systematic review and meta-analysis. Mol Biol Rep. 2012;39:9331–9338.

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

Table 1. Characteristics of study participants.

	SNPs analyses		DLPFC RNA expression analysis		DLPFC DNA methylation analyses		P-value
Variable	**Women**	**Men**	**Women**	**Men**	**Women**	**Men**	
Sample size	1711	651	738	323	420	222	
Age at baseline, years, Mean (SD)	78.0 (7.7)	77.4 (7.3)	80.9 (6.7)	79.1 (6.9)	81.5 (6.6)	78.9 (6.9)	F=32.5, p<0.001
Education, years, Mean (SD)	15.8 (3.4)	17.2 (4.0)	15.8 (3.2)	17.1 (3.9)	16.1 (3.4)	17.6 (3.6)	F=28.5, p<0.001
White non-Hispanic, n (%)	1559 (91)	615 (94)	724 (98)	318 (98)	407 (97)	216 (97)	χ^2=68.1, p<0.001
Mini Mental State Examination score at baseline, Mean (SD)	28.3 (1.8)	28.0 (2.1)	28.1 (1.8)	27.8 (2.2)	27.9 (1.9)	27.7 (2.3)	KW χ^2=46.4, p<0.001
Global Cognition at baseline, Mean (SD)	0.08 (0.53)	0.04 (0.54)	-0.02 (0.49)	0.00 (0.51)	-0.13 (0.48)	-0.07 (0.54)	F=13.8, p<0.001

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

Cognition status, n (%) No cognitive impairment Mild cognitive impairment Dementia	 1294 (76) 417 (24) 0	 467 (72) 184 (28) 0	 538 (73) 200 (27) 0	 225 (70) 98 (30) 0	 271 (65) 149 (35) 0	 141 (64) 81 (36)	χ^2=32.0, p<0.001
Years of follow up, Mean (SD)	9.7 (5.3)	9.7 (5.6)	9.0 (4.6)	9.0 (4.8)	7.2 (3.5)	7.2 (3.6)	F=25.8, p<0.001
Sample size of postmortem examinations	917	422	738	323	420	222	
Age at death, years, Mean (SD)	90.2 (6.5)	88.0 (6.5)	90.5 (6.5)	88.2 (6.5)	89.0 (6.6)	86.2 (6.2)	F=25.3, p<0.001
Years between last clinical evaluation and death, Mean (SD)	1.03 (1.59)	0.78 (0.95)	0.92 (1.30)	0.67 (0.75)	0.69 (0.66)	0.69 (0.80)	KW χ^2=17.6, p=0.004
Cognition status-last visit, n (%)							χ^2=14.9, p=0.135

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

No cognitive impairment	302 (33)	151 (36)	252 (34)	117 (36)	138 (33)	78 (35)	
Mild cognitive impairment	216 (24)	120 (28)	179 (24)	92 (28)	105 (25)	64 (29)	
Dementia	399 (43)	151 (36)	307 (42)	114 (35)	177 (42)	80 (36)	
Pathological diagnosis of Alzheimer's disease, n (%)	609 (66)	238 (56)	491 (67)	176 (54)	263 (63)	119 (54)	χ^2=34.1, p<0.001
Square root of Global Alzheimer's disease pathology score, Mean (SD)	0.79 (0.38)	0.67 (0.38)	0.79 (0.38)	0.66 (0.38)	0.77 (0.38)	0.65 (0.40)	F=14.9, p<0.001
Square root of Amyloid-β load, Mean (SD)	1.62 (1.13)	1.40 (1.13)	1.67 (1.14)	1.42 (1.17)	1.48 (1.07)	1.25 (1.12)	F=8.1, p<0.001
Square root of Tau tangle density, Mean (SD)	1.68 (1.30)	1.26 (1.07)	1.68 (1.28)	1.19 (0.97)	1.52 (1.15)	1.16 (1.02)	F=18.8, p<0.001
Lewy bodies, n (%)	223 (24)	116 (27)	179 (24)	93 (29)	98 (23)	53 (24)	χ^2=5.0, p=0.411

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

TDP-43 in hippocampus or neocortex, n (%)	308 (34)	98 (23)	240 (33)	75 (23)	122 (29)	46 (21)	χ^2=28.1, p<0.001
Hippocampal sclerosis, n (%)	91 (10)	30 (7)	68 (9)	26 (8)	32 (8)	14 (6)	χ^2=5.8, p=0.323
One or more Macroinfarcts, n (%)	319 (35)	155 (37)	254 (34)	118 (37)	137 (33)	83 (37)	χ^2=2.6, p=0.759
One or more microinfarcts, n (%)	271 (30)	134 (32)	208 (28)	98 (30)	104 (25)	61 (27)	χ^2=6.1, p=0.298
Moderate to severe atherosclerosis, n (%)	291 (32)	133 (32)	233 (32)	102 (32)	186 (44)	98 (44)	χ^2=36.9, p<0.001
Moderate to severe arteriolosclerosis, n (%)	303 (33)	107 (25)	246 (33)	85 (26)	166 (40)	74 (33)	χ^2=25.9, p<0.001
Moderate to severe cerebral amyloid angiopathy, n (%)	325 (35)	145 (34)	263 (36)	104 (32)	143 (34)	70 (32)	χ^2=2.6, p=0.761

P-values were derived from analysis of variance, chi-square, and Kruskal-Wallis tests comparing the 6 different subsets in continuous, categorical, and rank data, respectively. Degree of freedom in all the analyses was 5.

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

Table 2. Summary of associations of the 3 estrogen receptor (*ER*) genes with longitudinal changes of cognition and underlying pathologies in older women.

ER	**Variant**		**Cognition**		**AD pathologic indices**			**Non-AD pathologies**							
			Level	**Rate of Decline**	Aβ	**Tau**	**Global AD Score**	**Lewy Body**	**TDP-43**	**HS**	**Macro-infarct**	**Micro-infarct**	**Athero-sclerosis**	**Arteriolo-Sclerosis**	**CAA**
GPER 1	**SNPs**		**0.028**	NS	NS	NS	NS	NS	NS	NS	NS	**0.038**	NS	NS	NS
	DNA methylation		**<0.001**	**<0.001**	NS	**0.013**	**0.013**	NS	NS	NS	NS	NS	NS	NS	NS
	RNA	**DLP**	**-0.181 (0.045)**	**-0.014 (0.004)**	0.065 (0.046)	**0.140 (0.051)**	0.028 (0.015)	NS	NS	NS	NS	NS	NS	NS	NS

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

			<0.001	**0.005**	0.240	**0.039**	0.139								
		PCC	**-0.201 (0.076) 0.026**	-0.015 (0.007) 0.083	0.118 (0.083) 0.240	0.134 (0.096) 0.327	0.047 (0.029) 0.152	NS	NS	NS	NS	NS	NS	NS	NS
		ACC	-0.124 (0.102) 0.341	-0.012 (0.009) 0.307	0.069 (0.110) 0.637	-0.045 (0.112) 0.817	0.014 (0.036) 0.696	NS	NS	NS	NS	NS	NS	NS	NS
ER2	**SNPs**		**0.009**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	DNA methylation		**<0.001**	**<0.001**	NS	**0.013**	NS	NS	NS	NS	NS	NS	NS	NS	NS

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

		DLP	-0.047 (0.057) 0.493	-0.005 (0.005) 0.388	0.125 (0.057) 0.091	0.041 (0.064) 0.782	**0.048 (0.019) 0.035**	NS	NS	NS	NS	NS	NS	NS	NS
	RNA	**PCC**	-0.166 (0.078) 0.068	-0.015 (0.007) 0.083	0.184 (0.085) 0.091	0.174 (0.099) 0.237	**0.074 (0.029) 0.035**	NS	NS	NS	NS	NS	NS	NS	NS
		ACC	0.018 (0.097) . 0.857	0.005 (0.009) 0.701	0.028 (0.103) 0.783	0.024 (0.105) 0.817	0.036 (0.034) 0.358	NS	NS	NS	NS	NS	NS	NS	NS
ER1	**SNPs**		**<0.001**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	DNA		**<0.001**	**<0.001**	NS	**0.016**	NS	NS	NS	N	NS	NS	NS	NS	NS

ACCEPTED

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

	methylatio n								S					

Cells with single values indicate q-values (FDR-corrected p-values) derived from omnibus tests that combine p-values of the associations of SNPs or CpG sites with the outcomes. Cells with 3 values indicate estimate (SE), q-value (FDR corrected p-value) derived from mixed effects models (cognition as the outcome) or linear regression models (AD pathology indices as the outcomes). Bold cells indicate significant associations.

NS: Not significant; HS: Hippocampal sclerosis; CAA: Cerebral amyloid angiopathy; DLP: Dorsolateral prefrontal cortex; ACC: Anterior cingulate cortex; PCC: Posterior cingulate cortex.

ACCEPTED

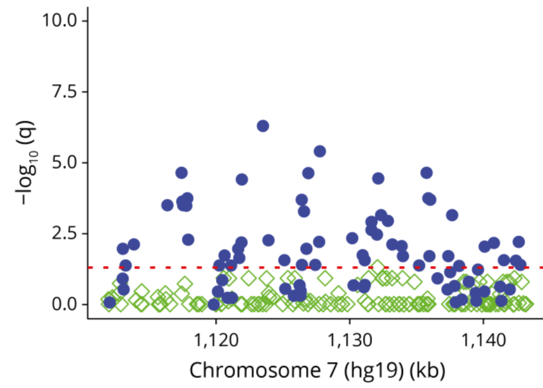
Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

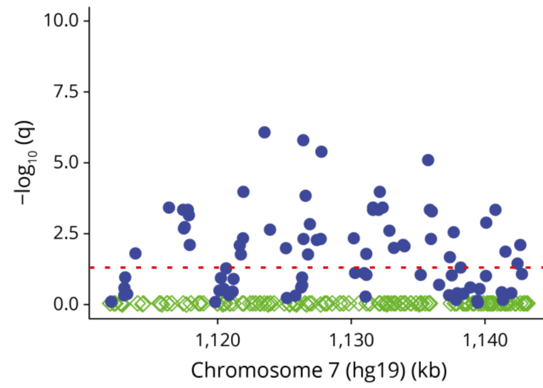
Figure 1. Association of polymorphisms and DNA methylation at estrogen receptor genes, *ER1*, *ER2*, and G*PER1*, with level of global cognition and rate of cognitive decline.

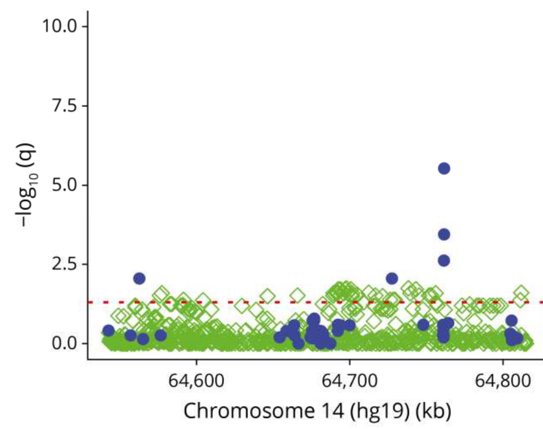
In each plot, the association of all SNPs (green circles) and CpG sites (Blue circles) in the examined genetic region with level of global cognition (A) or rate of cognitive decline (B) is illustrated. X-axis indicates the chromosomal position of the SNPs/CpG sites and the Y-axis indicates the FDR-corrected P-value of the association between the SNP/CpG site and the outcome. The horizontal red dashed lines show the level above which the associations are statistically significant.

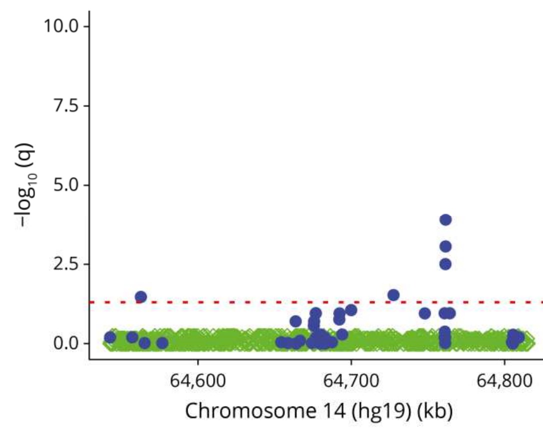
ACCEPTED

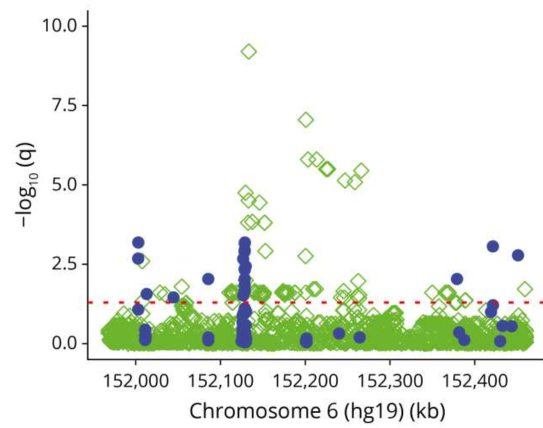
Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

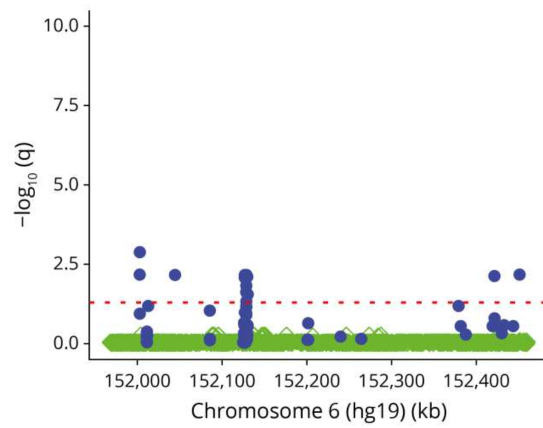






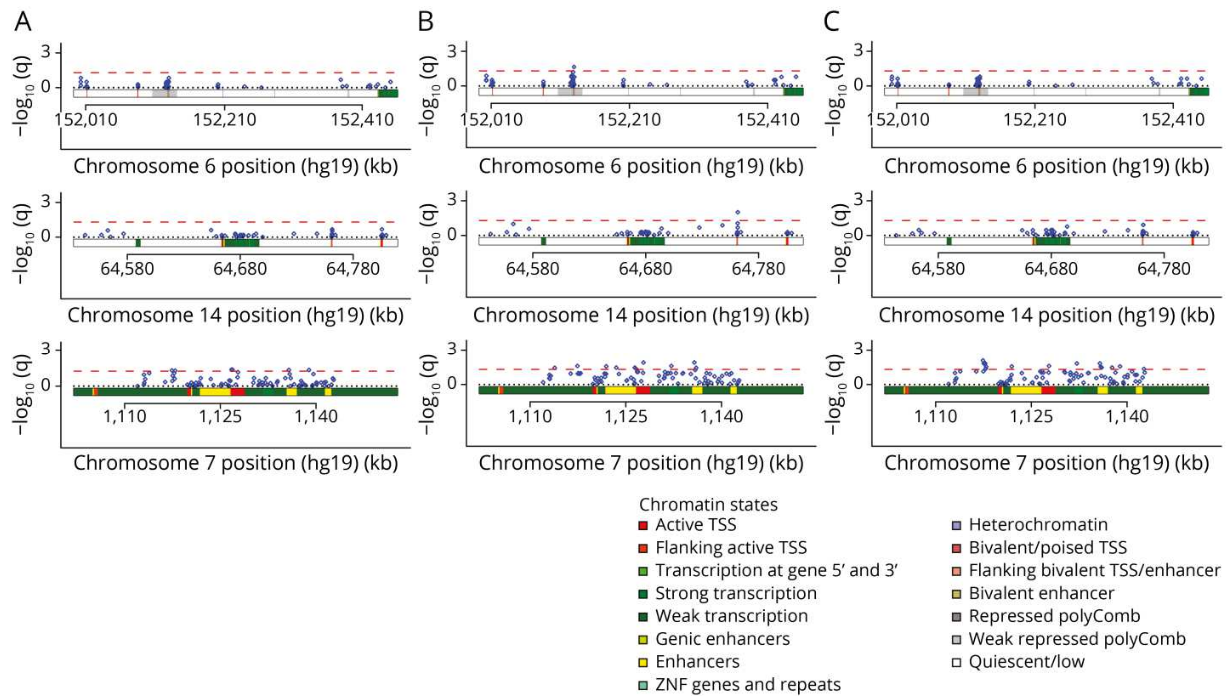






Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

Figure 2. Association of DNA methylation at estrogen receptor genes with Alzheimer's disease pathology indices.



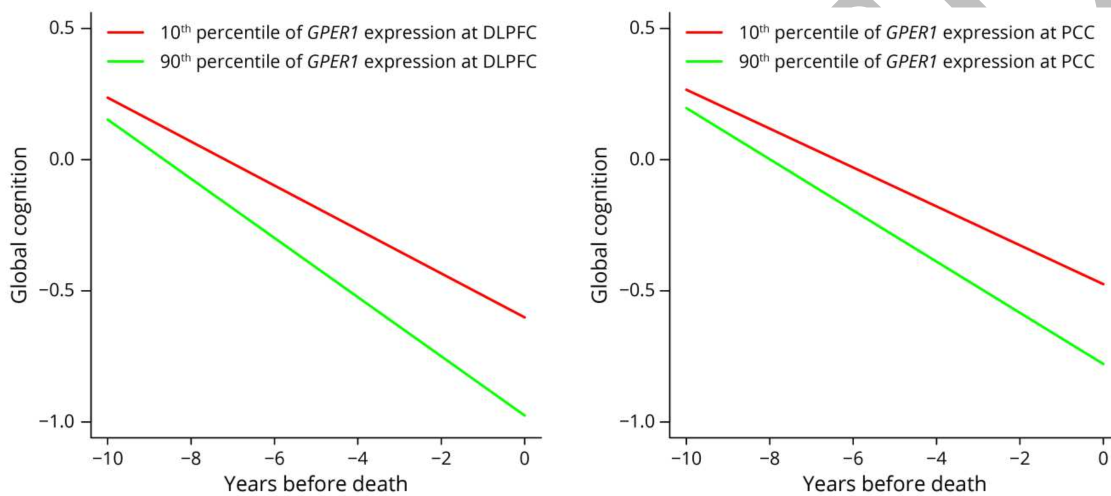
In each plot, the association of individual CpG sites in the examined genetic region with the level of amyloid-β load (A), tau tangle density (B), or global AD pathology score (C) is illustrated, derived from linear regression. The Y-axis indicates the FDR-corrected P-value of the association and the X-axis illustrates the location of the CpG site in the genome. The color bands in the X-axis represent the chromatin state of the genetic region.

A. Amyloid-β load score B. Tau tangle density C. Global AD Pathology

Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

Figure 3. Association of *GPER1* expression level with cognitive decline prior to death.

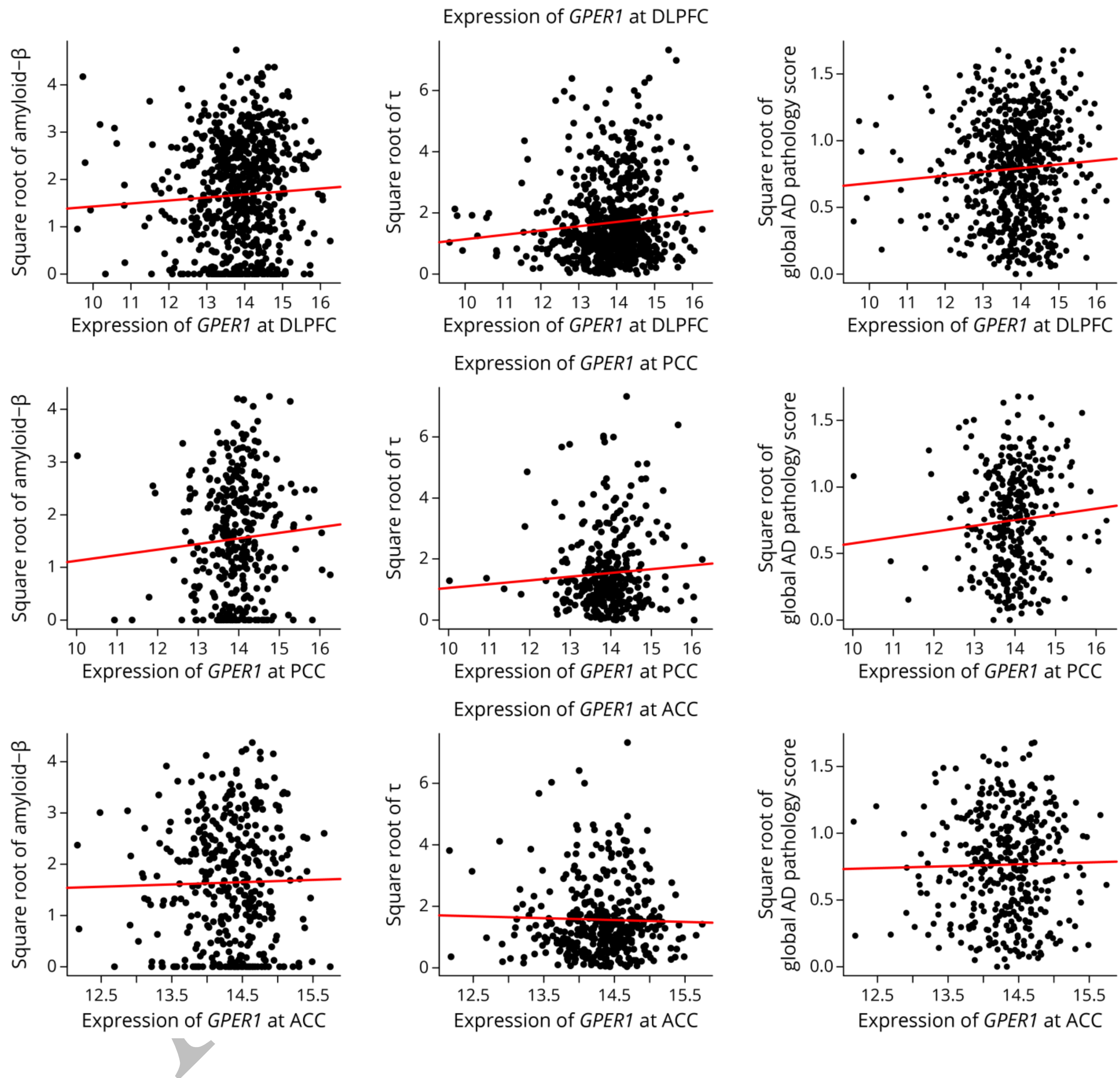
In each plot, the association of expression level of *GPER1* at DLPFC (left panel), or at PCC (right panel) with cognitive decline in the last 10 years before death is illustrated. Each plot illustrates cognitive trajectory in an average 90-year-old woman with 16 years of education with either low (10th percentile) or high (90th percentile) expression level of the *ER* gene. DLPFC: dorsolateral prefrontal cortex; PCC: posterior cingulate cortex.



ACCEPTED

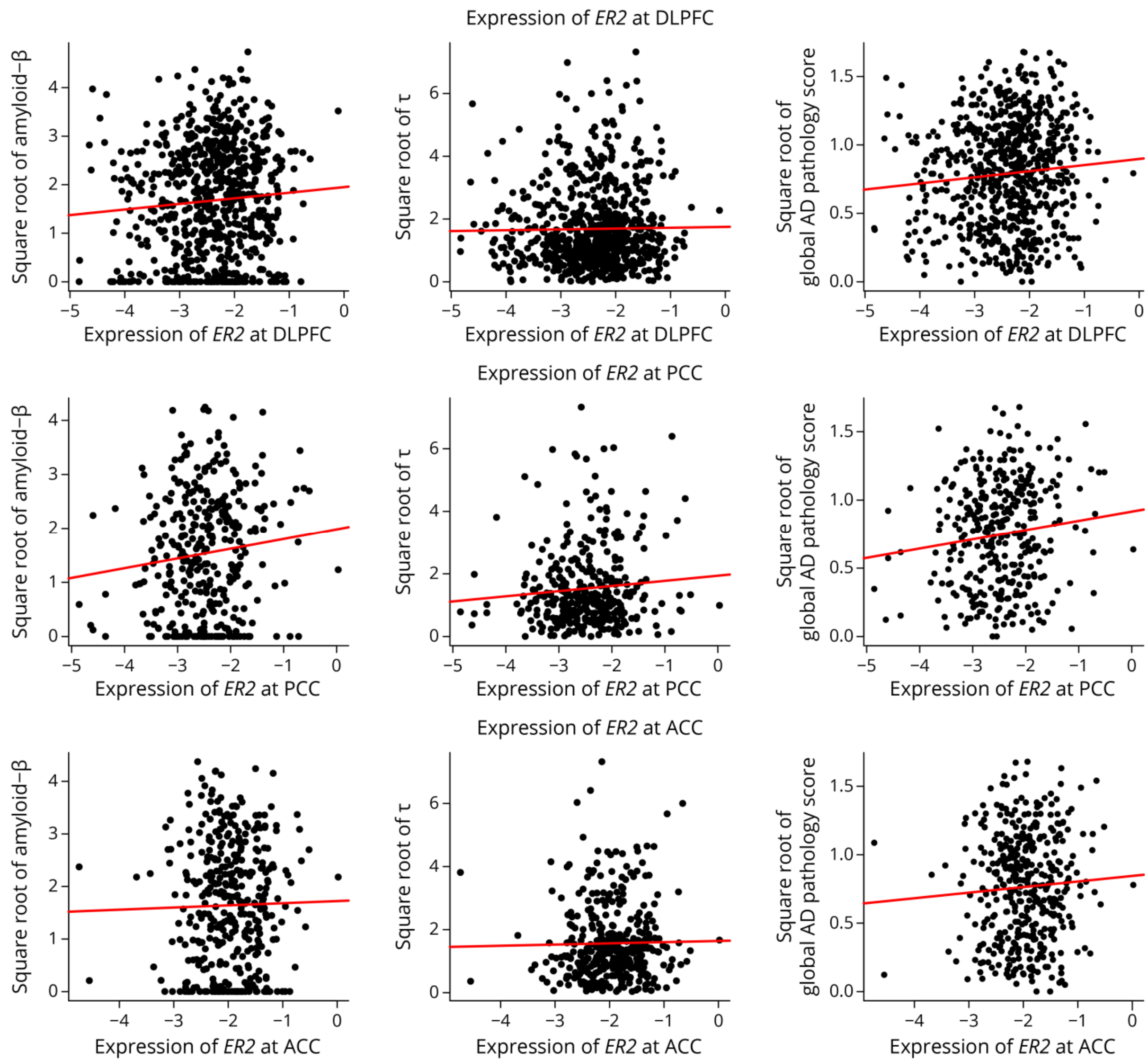
Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

Figure 4. Association of *GPER1* expression levels at dorsolateral prefrontal cortex (DLPFC), posterior cingulate cortex (PCC), and anterior cingulate cortex (ACC) with AD pathology indices.



Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

Figure 5. Association of *ER2* expression levels at dorsolateral prefrontal cortex (DLPFC), posterior cingulate cortex (PCC), and anterior cingulate cortex (ACC) with AD pathology indices.



Copyright © 2023 American Academy of Neurology. Unauthorized reproduction of this article is prohibited

Neurology®

Estrogen Receptor Genes, Cognitive Decline, and Alzheimer Disease
Shahram Oveisgharan, Jingyun Yang, Lei Yu, et al.
Neurology published online January 25, 2023
DOI 10.1212/WNL.0000000000206833

This information is current as of January 25, 2023

Updated Information & Services	including high resolution figures, can be found at: http://n.neurology.org/content/early/2023/01/25/WNL.0000000000206833.full
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): **Alzheimer's disease** http://n.neurology.org/cgi/collection/alzheimers_disease **Cluster headache** http://n.neurology.org/cgi/collection/cluster_headache **Cohort studies** http://n.neurology.org/cgi/collection/cohort_studies **Gene expression studies** http://n.neurology.org/cgi/collection/gene_expression_studies **Inclusion, Diversity, Equity, Anti-racism, and Social Justice (IDEAS)** http://n.neurology.org/cgi/collection/all_equity_diversity_and_inclusion
Permissions & Licensing	Information about reproducing this article in parts (figures,tables) or in its entirety can be found online at: http://www.neurology.org/about/about_the_journal#permissions
Reprints	Information about ordering reprints can be found online: http://n.neurology.org/subscribers/advertise

Neurology ® is the official journal of the American Academy of Neurology. Published continuously since 1951, it is now a weekly with 48 issues per year. Copyright © 2023 American Academy of Neurology. All rights reserved. Print ISSN: 0028-3878. Online ISSN: 1526-632X.