Sex-Specific Genetic Predictors of Alzheimer's Disease Biomarkers

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Abstract

Cerebrospinal fluid (CSF) levels of amyloid-β 42 (Aβ42) and tau have been evaluated as endophenotypes in Alzheimer's disease (AD) genetic studies. Although there are sex differences in AD risk, sex differences have not been evaluated in genetic studies of AD endophenotypes. We performed sex-stratified and sex interaction genetic analyses of CSF biomarkers to identify sex-specific associations. Data came from a previous genome-wide association study (GWAS) of CSF Aβ42 and tau (1527 males, 1509 females). We evaluated sex interactions at previous loci, performed sex-stratified GWAS to identify sex-specific associations, and evaluated sex interactions at sex-specific GWAS loci. We then evaluated sex-specific associations between prefrontal cortex (PFC) gene expression at relevant loci and autopsy measures of plaques and tangles using data from the Religious Orders Study and Rush Memory and Aging Project. In Aβ42, we observed sex interactions at one previous and one novel locus: rs316341 within *SERPINB1* (p=0.04) and rs13115400 near *LINC00290* (p=0.002). These loci showed stronger associations among females (β=−0.03, p=4.25×10^{-8}; β=0.03, p=3.97×10^{-8}) than males (β=−0.02, p=0. 009; β=0.01, p=0.20). Higher levels of expression of *SERPINB1, SERPINB6, and SERPINB9* in PFC was associated with higher levels of amyloidosis among females (corrected p-values<0.02) but not males (p>0.38). In total tau, we observed a sex interaction at a previous locus, rs1393060 proximal to *GMNC* (p=0.004), driven by a stronger association among females (β=0.05, p=4.57×10^{-10}) compared to males (β=0.02, p=0.03). There was also a sex-specific association between rs1393060 and tangle density at autopsy (p_{female}=0.047; p_{male}=0.96), and higher levels of expression of two genes within this locus were associated with lower tangle density among females (*OSTN* p=0.006; *CLDN16* p=0.002) but not males (p 0.32). Results suggest a female-specific role for *SERPINB1* in amyloidosis and for *OSTN* and *CLDN16* in tau pathology. Sex-specific genetic analyses may improve understanding of AD's genetic architecture.

Keywords

Alzheimer disease; cerebrospinal fluid biomarkers; neuropathology; sex difference; APOE; amyloid; tau

Introduction

Two-thirds of all prevalent Alzheimer' disease (AD) cases are female [37, 38], and emerging evidence has highlighted striking sex differences in the genetic drivers [19], clinical severity [25], and neuropathological presentation of AD [2, 4, 28]. The *Lancet Neurology* Commission thus asserted that a focus on sex differences in AD is essential to advance the field [37]. The identification of sex-specific AD genetic drivers could transform the way treatments are developed and administered and be a critical step towards personalized interventions for AD.

AD is characterized by plaques consisting of aggregated amyloid β (Aβ) and neurofibrillary tangles composed of phosphorylated tau protein [47]. Cerebrospinal fluid (CSF) Aβ42 is a biomarker for brain amyloidosis, while total tau (t-tau) reflects the intensity of neurodegeneration and phosphorylated tau (p-tau) relates to tau pathology [7]. Alterations in both of these proteins are measurable in cerebrospinal fluid (CSF) years prior to the clinical onset of disease [31, 32], and have become a focus of the preclinical characterization of AD [30]. Work over the past five years has sought to elucidate the genetic architecture of CSF amyloid and tau through genome-wide association studies (GWAS) [11, 12, 15]. *APOE* has a strong association with both CSF amyloid and tau. Recent work from our group [26] and others [1] suggests that the *APOE* association with t-tau and p-tau is stronger among females compared to males, but there is no sex difference in associations between *APOE* and amyloidosis. There have been two additional loci identified in relation to amyloidosis, and four related to CSF tau [15]. Yet, sex differences have not been integrated into GWAS studies of CSF AD biomarkers.

This manuscript provides a comprehensive characterization of sex-specific genetic predictors of CSF amyloid and tau. The use of quantitative traits (in this case continuous measures of CSF Aβ42, t-tau, and p-tau) provides additional statistical power and outcome metrics that are more proximal to genetic function. We perform a sex-stratified GWAS of CSF amyloid and tau to identify loci that show disparate associations between males and females. For all sex-specific genome-wide associations, we formally assess whether the association differs by sex. Finally, we leverage transcriptomic data from human prefrontal cortex (PFC) tissue to test whether we observe sex-specific associations between gene expression at the relevant loci and levels of AD neuropathology at autopsy. This work highlights sex-specific markers of amyloid and tau and clarifies the degree to which known loci act in a sex-specific manner.

Methods

Data used here were previously described [15]. Data were acquired from seven studies of cognitive aging, all of which included lumbar puncture and clinical assessments. The quantification of CSF biomarker levels and clinical assessments were completed by each site independently. The Alzheimer Disease Neuroimaging Initiative (ADNI) is a longitudinal, multi-site observational study including AD, mild cognitive impairment (MCI), and elderly cognitively normal controls [42, 46]. The Alzheimer's Disease Genetics Consortium (ADGC) was formed as a collaboration to utilize the collective resources of the AD research community to conduct GWAS to identify genes associated with late-onset AD risk; data were obtained from the Mayo Clinic (MAYO), University of Pennsylvania (UPENN), and BIOCARD as part of this collaboration [40]. Data from AD cohorts in Sweden were obtained from a multi-center study designed to determine diagnostic accuracy of CSF Aβ42, t-tau, and p-tau [36]. The University of Washington (UW) provided data from a CSF AD biomarker study [33]. Detailed information about the design of each study has been described previously by the ADGC [40], the ADNI [42, 46], Knight ADRC [17], UW [33], or Mattsson et al [36]. Clinical and demographic characteristics of the sample are presented by cohort in suppl. table 1 (Online Resource 2).

Ethics Statement

The Institutional Review Boards of all participating institutions approved the study and research was carried out in accordance with the approved protocols. Written informed consent was obtained from participants or their family members. Secondary analyses of all data were approved by the Vanderbilt University Medical Center IRB.

Genotyping and QC

As previously reported [15], genotyping was completed by each study and imputed using 1000 Genomes Project Phase 3 data. Prior to imputation, all genotype data were processed through the same quality control (QC) protocol, including removing single nucleotide polymorphisms (SNPs) with a poor call rate (<98%) or outside of Hardy-Weinberg equilibrium ($p < 1 \times 10^{-6}$). Samples were excluded for sex inconsistency (genetically observed compared to self-reported sex) or cryptic relatedness (Pihat 0.25). *APOE* was directly genotyped using a Taqman assay [15]. Imputation was completed using IMPUTE2 (version 2.3.2) and genotyping calls were used for all genotypes with a probability of 90%. Imputed genotypes were excluded with minor allele frequency (MAF) <2% or information score<0.30. Principal component analyses were conducted and, based on visual inspection of principal component graphs, only individuals that clustered with the European ancestry population from the 1000 Genomes Project were included in the study.

Quantification of Biomarker Outcomes

Harmonization procedures for the CSF analyses have been described [15]. Briefly, raw values were log-transformed within each study and centered using the study mean. This normalization approach has been leveraged previously [15] and is an effective approach to minimize the influence of study differences in assays used to measure the CSF analytes. For the present analyses we excluded one small dataset from the original analysis, the Saarland University sample from Hamburg (HB), which did not include Clinical Dementia Rating (CDR) or diagnosis and did not include all CSF measures. Our decision to drop HB (n=105) was primarily driven by visual inspection of outcome distributions that revealed an inequivalent variance structure in HB relative to the other datasets. Secondary analyses were performed that check for study specific effects including meta-analysis and the comparison of regression coefficients across datasets.

Autopsy Measures of Gene Expression and Neuropathology

We leveraged data from the Religious Orders Study/Memory and Aging Project (ROS/MAP) through the Accelerating Medicines Partnership AD project (https://www.synapse.org/#! Synapse:syn2580853/wiki/). ROS began in 1994 and involves older Catholic nuns, priests, and brothers recruited from across the US. MAP began in 1997 and involves older lay persons recruited from retirement communities, subsidized housing facilities, and social service agencies in the Chicago metropolitan area. Participants in ROS/MAP enrolled without dementia and agreed to annual clinical evaluations and organ donation [5, 6]. RNA expression levels were obtained from frozen sections of the dorsolateral prefrontal cortex (PFC) that were manually dissected from postmortem brain tissue. Details of RNA extraction, processing, QC, and normalization have been published [34]. RNA was isolated

using the RNeasy lipid tissue kit (Qiagen, Valencia, CA) and was reverse transcribed and biotin-UTP labeled using the llumina® TotalPrep™ RNA Amplification Kit from Ambion (Illumina, San Diego, CA). Expression signals were generated using the Beadstudio software suite (Illumina, San Diego, CA). Standard control and normalization methods were employed to account for technical variability due to differences in hybridization dates. For the present analyses, low abundance genes (expressed in <10% of the cohort) were filtered out from analyses to reduce confounding due to floor effects.

Amyloid-β load and tau tangles were measured using immunohistochemistry and quantified by image analysis and stereology [5, 6]. For amyloid-β load, we used levels of Aβ measured in 8 cortical regions of the brain [5, 6]. For tangle density, we used levels of abnormally phosphorylated tau measured with AT8 antibody across 8 brain regions [5, 6]. 362 males and 704 females had both neuropathology and SNP data available for analysis, and 207 males and 374 females had both neuropathology and gene expression data available.

Posthoc Analyses of Memory Performance, Executive Function Performance, and Hippocampal Volume

Genome-wide associated SNPs were further evaluated for associations with memory, executive function, and hippocampal volume using ADNI data. Composite measures of memory and executive function have been quantified previously [10, 22], and hippocampal volumes were quantified using FreeSurfer [13, 20, 21] as previously described [39]. ICV-adjusted hippocampal volumes were generated from residuals of a linear regression between raw volumes and ICV among cognitively normal participants at baseline [50].

Statistical Analyses

Statistical analyses were completed using PLINK (Version 1.9, https://www.cog-genomics.org/plink/1.9) and RStudio (Version 1.0.136; https://www.rstudio.com/). Additive genetic coding was used for all analyses, and all analyses included covariates for age (at CSF acquisition), cohort, and the first two population principal components. Sex-specific analyses were then evaluated using the same covariates stratifying by males and females. For sex-interaction analyses a sex x SNP interaction term was included in the original statistical model.

To identify sex differences at known loci, we re-evaluated the most significant single-SNP associations from seven genome-wide significant loci for Aβ42, t-tau, and p-tau (p-tau$_{181}$ specifically) that had been published [15]. We noted any loci with a significant sex-interaction effect (nominal p<0.05) on the published outcome and corrected for the seven SNPs evaluated. Next, to identify novel sex-specific loci, we assessed all GWAS markers for associations within one sex using the established GWAS threshold for statistical significance ($\alpha=5\times10^{-8}$). All significant sex-stratified effects were also assessed for sex interactions to test whether the coefficients differed by sex. Sex-stratified Miami plots were generated using EasyStrata (version 16.0) [51]. Genomic inflation factors for the genome-wide association analyses ranged from $\lambda=1.00–1.03$ (suppl. figure 1 [Online Resource 1]).

Sex-specific loci identified in candidate and genome-wide analyses were further evaluated for functional significance. Expression quantitative trait loci (eQTL) analyses were

completed using published gene expression data from Braineac (http://www.braineac.org/) and the Genotype-Tissue Expression project (GTEx; www.gtexportal.org) [23, 24, 35]. For eQTL association analyses, we corrected for the total number of tissue-gene combinations using the false discovery rate (FDR) procedure.

Additional analyses were completed using the measures outlined above. We evaluated SNP associations with the relevant neuropathology covarying for age at death and education. Next, we tested sex-stratified and sex interaction associations between PFC expression of genes implicated in eQTL analyses and the neuropathology of interest, correcting for multiple comparisons using the FDR procedure. If Braineac and GTEx did not provide strong eQTL evidence at a given locus, we evaluated all genes within the cis region of the locus (i.e., 1 MB upstream and downstream of the SNP) and corrected for multiple comparisons using the FDR procedure.

To explore if genome-wide associated SNPs were correlated with biomarkers of AD risk and progression, we tested for associations with adjusted hippocampal volume, episodic memory performance, and executive function performance in ADNI participants at baseline and over time. Linear regression covarying for age at baseline was used for cross-sectional analyses. Mixed-effects linear regression was used for longitudinal analyses. Fixed effects included age at baseline, SNP, interval (modeled as time in years from baseline), and a SNP x interval interaction term. Random effects included the intercept and time interval. All analyses were run in the entire ADNI cohort (males and females), in males only, in females only, and in the entire cohort including a SNP x sex interaction. Additionally, sex-stratified and sex interaction results were also calculated from a recently published age-of-onset analysis of AD [29]. Raw genotype data were only available from GERAD and ADGC, so we restricted analyses to these two groups. Survival analysis followed the original procedures first stratified by males and females and second testing SNP x sex interactions.

Role of the Funding Source

The funders of the study had no role in the collection, analysis, or interpretation of data; in the writing of the report; or in the decision to submit the paper for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Clinical and demographic characteristics of the sample are presented by sex in Table 1 and by cohort in suppl. table 1 (Online Resource 2). In the combined sample across cohorts, females were younger (p<0.001), had lower levels of CSF amyloid than males (p=0.01), and were less likely to have a diagnosis of AD (p<0.001) compared to males. Males and females did not differ in levels of normalized CSF t-tau, normalized CSF p-tau, or *APOE* ε4 carrier status (p-values>0.10), but did differ on CSF Aβ42 (p=0.01). Given the difference in age, secondary analyses were performed matching for age and stratifying by diagnosis. The sex difference in CSF Aβ42 was not present in the age-matched subgroup (p=0.18). Methodology, participant characteristics, and detailed results for age-matched analyses are

presented in suppl. methods (Online Resource 1) and suppl. tables 2–11 (Online Resource 2).

Sex Differences for the Top SNP at Previously Published Loci

Sex interaction and sex-stratified results for the SNPs with the strongest reported associations at published GWAS loci are presented in Table 2. For Aβ42 analyses, we observed a significant interaction between sex and rs316341 on chromosome 6 (p=0.04, corrected p=0.28), whereby the association was genome-wide among females (p=4.25×10^{-8}, corrected-p<0.001) compared to a nominal association among males (p=0.009, corrected-p=0.06). The association between rs316341 and Aβ42 was observed in age-matched cognitively normal controls (females: n=615, β=−0.04, p=0.0002; males: n=615, β=−0.03, p=0.001) but did not reach statistical significance in cases (females: n=613, p=0.14; males: n=613, p=0.42). In t-tau analyses, the previously identified locus on chromosome 3 (rs35055419) showed a genome-wide association among females (p=2.57×10^{-8}, corrected-p<0.001) but a nominal association among males (p=0.0003, corrected-p=0.002). However, the sex x SNP interaction did not reach statistical significance (p=0.17, corrected-p=1). Nominal association between rs35055419 and t-tau was observed in age-matched controls (females β=0.04, p=3.75×10^{-4}; males β=0.03, p=0.02) and cases (females β=0.03, p=0.04; males β=0.04, p=0.003).

We did not observe a significant sex interaction between SNPs in the *APOE* locus and any of the CSF biomarkers (p>0.10, suppl. table 12 [Online Resource 2]). We have previously published a sex x *APOE*-ε4 interaction on CSF t-tau and p-tau levels [26], and when meta-analyzing across cohorts here we also observed a sex difference in the association between *APOE*-ε4 and t-tau (p=0.008). Further, when meta-analyzing across the datasets included here (evaluating the *APOE* locus determined by Taqman genotyping of rs7412 and rs429358) and the non-overlapping datasets previously published, we confirmed a sex x *APOE*-ε4 interaction on both t-tau (β=0.21, p=0.00002) and p-tau (β=0.13, p=0.01). In eight out of the nine datasets analyzed across the two studies, the point estimate for the *APOE*-ε4 association with CSF t-tau was larger among females compared to males (suppl. figure 2 [Online Resource 1]).

Genome-Wide Sex-Specific Associations

Significant sex-stratified GWAS loci (outside *APOE*) are presented in Table 3. In CSF Aβ42 analyses (Figure 1; suppl. tables 6 and 7 [Online Resource 2]) rs13115400 on chromosome 4 was significant among females (p=4.61×10^{-8}, corrected-p=0.046), but not among males (p=0.19, interaction p=0.003, corrected-p-values=1.0). As highlighted above, one previous SNP on chromosome 6, rs316341, also showed a genome wide significant association with CSF Aβ42 among females but not males. In CSF t-tau analyses (Figure 2; suppl. tables 8 and 9 [Online Resource 2]), a SNP within the same chromosome 3 locus previously identified by Deming et al. [15] (near *GMNC*) was associated among females (rs1393060, p=8.27×10^{-10}, corrected-p=0.0008), with only a nominal association among males (p=0.03, interaction p=0.004, corrected-p-values=1). The female-specific association was also present when evaluating age-matched controls (females β=0.04, p=0.0008; males β=0.01, p=0.26), but was more comparable among age-matched cases (females β=0.04, p=0.0004; males

β=0.03, p=0.04). Locus zoom plots for CSF Aβ42 and t-tau are presented in suppl. figures 3 and 4 (Online Resource 1). There were no sex-specific associations with p-tau that met genome-wide significance ($p<5\times10^{-8}$) with the exception of the *APOE* locus (suppl. figure 5 [Online Resource 1], suppl. tables 10 and 11 [Online Resource 2]).

We also analyzed each dataset separately and performed meta-analyses for the three loci identified in candidate and GWAS analyses. The directions of effect for all three identified SNPs were consistent within sex and across studies analyzed separately. Results from meta-analyses were consistent with the joint analysis (suppl. figures 6–8 [Online Resource 1]). Age-matched results are presented in suppl. tables 6–11 (Online Resource 2), and estimates for GWAS SNPs remain consistent, suggesting observed associations are not driven by age differences.

eQTL Results for Sex-Specific Associations

For Aβ42, one previously identified SNP (rs316341) and one novel SNP (rs13115400) showed sex-specific associations and were further evaluated for eQTL associations. For rs316341, significant eQTL associations across all 10 brain tissues in Braineac were seen with *SERPINB1, SERPINB6,* and *SERPINB9* (aveALL p-values<0.0012, FDR-corrected aveALL p-values=0.047; suppl. table 13 [Online Resource 2]). This eQTL signal appears to be driven by gene expression in the hippocampus ($p=4.3\times10^{-5}$, corrected-p[*all tissue gene combinations*]=0.13; 6.5×10^{-5}, corrected-p=0.20 for *SERPINB1* and *SERPINB9*, respectively). Similarly in GTEx, significant eQTL associations were observed with *SERPINB1* expression in brain cerebellar hemisphere ($p=4.9\times10^{-6}$, corrected-p=0.013) and transformed fibroblasts ($p=1\times10^{-5}$, corrected-p=0.013) and with SERPINB9 pseudogene 1 (*RP11–420G6.4*) expression in the cortex of the brain ($p=1.4\times10^{-6}$, corrected-p=0.005; suppl. table 14 [Online Resource 2]). No significant eQTL associations for rs13115400 were observed in Braineac (suppl. table 15 [Online Resource 2]) or GTEx.

For t-tau, the top female-specific association within the chr. 3 locus was rs1393060 and was further evaluated for eQTL associations. In Braineac, we did not observe any eQTL association that survived correction for multiple comparisons. However, the strongest observed association was between rs1393060 and osteocrin (*OSTN)* expression in the frontal cortex (p=0.00057, corrected-p=0.86; suppl. table 16 [Online Resource 2]). No significant eQTL associations were observed in tissues within the GTEx database.

Autopsy Validation and Extension of Sex-Specific Effects

The three identified SNPs with potential sex-specific effects on CSF biomarker levels (rs1393060, rs13115400, and rs316341) were tested for sex differences in AD neuropathology. None of the three SNPs had a significant sex interaction (Table 4), but rs1393060 did show evidence of an association with tau density at autopsy among females (p=0.047, corrected-p=0.14) but not males (p=0.96, corrected-p=1; Table 4).

Given the strong eQTL evidence for rs316341 on *SERPINB1, SERPINB6,* and *SERPINB9*, PFC expression of these three genes was further assessed for sex-specific associations with amyloid burden (suppl. table 17 [Online Resource 2]). Consistent with SNP results, we observed associations between expression levels of *SERPINB1* (β[SE]=0.08[0.03], p=0.010,

corrected-p=0.011), *SERPINB6* (β=0.02[0.004], p=0.0002, corrected-p=0.0004; Figure 3) and *SERPINB9* (β[SE]=0.17[0.07], p=0.011, corrected-p=0.011) with amyloid-β load among females that were not observed among males (p-values>0.43). Sex interactions were not statistically significant.

There was not strong eQTL evidence at the rs1393060 locus, so we evaluated all 18 genes in cis that were measured in ROS/MAP autopsy samples. Of these 18 genes, four genes showed an association with tangle density across sexes, including *RP11–1976K.*1, *CLDN16*, *GMNC*, and *OSTN* (suppl. table 18 [Online Resource 2]). Interestingly, both *CLDN16* and *OSTN* showed a significant association among females (corrected-p-values=0.040), but not among males (p-values 0.32). As displayed in Figure 4, higher levels of both *OSTN* and *CLDN16* were associated with lower tangle density among females.

We did not observe strong eQTL evidence at the rs13115400 locus, so we evaluated all five cis genes available for analysis (suppl. table 19 [Online Resource 2]). Three genes showed significant associations, two of which were associated just in females (*AC108142.1* and *TENM3*, corrected-p 0.0018) and one just in males (*RP11–433O3.1*, corrected-p=0.0074). There were no significant sex-interactions.

Results from the posthoc analyses of hippocampal volume, memory, and executive function are presented in suppl. table 20 (Online Resource 2). In both males and females combined, we observed an association between rs1393060 and both memory (β=−0.08, p=0.045) and executive function (β=−0.12, p=0.011) performance but did not observe any sex-specific associations. Results from posthoc survival analyses using age-of-onset data from IGAP are presented in suppl. table 21 (Online Resource 2). In the ADGC, rs1393060 and rs316341 were significantly associated among females (p<0.01), but not males (p>0.1). A significant sex interaction was observed for rs316341 (p=0.009). No associations were observed in the GERAD dataset.

Discussion

We evaluated sex specific genetic associations with biomarkers of AD neuropathology measured in CSF. We observed female-specific associations with amyloid levels in one previously identified locus on chromosome 6 and one novel locus on chromosome 4, both of which did not show an association among males. For t-tau, we confirmed that the *APOE* association is stronger among females compared to males [26] and provide new evidence that the previously observed t-tau association on chromosome 3 is driven by females. Finally, we provide functional evidence that the sex-specific association on chromosome 6 is driven by a female-specific effect of *SERPINB1* gene expression levels on amyloidosis, and that the sex-specific association at chromosome 3 may be driven by a female-specific association between *OSTN* or *CLDN16* expression on t-tau levels. Together, our results highlight the importance of sex considerations in models of AD risk, suggesting potential candidate pathways that may differentially drive AD neuropathology among males and females.

We provide evidence that genetic variation within Serpin Family B Member 1 (*SERPINB1)* is related to amyloidosis, particularly among females. A similar sex-specific association was also observed in posthoc age-of-onset analyses, in which the minor allele of rs316341 was associated with an earlier age-of-onset among females, but not males. Our eQTL and PFC expression results provide support for *SERPINB1*'s potential role in amyloidosis, particularly among females. Serpins are a family of protease inhibitors that are expressed ubiquitously including all cell types of the brain. Serpins have been implicated as potential inhibitors of Aβ toxicity [48], likely through a role in regulating neutrophil infiltration [18] in response to amyloidosis [15]. Neutrophil response is sex dimorphic and modulated by sex hormones in rats [14], and there is evidence that estrogen has direct effects on neutrophil infiltration and clearance [43]. In total, our results suggest that *SERPINB1* may be associated with amyloidosis in a sex-specific manner, with evidence suggesting it is a particularly relevant predictor among females. The previous work on *SERPINB1* and neutrophil signaling highlights the need to better understand how gonadal hormonal changes in late life may impact the innate immune response to amyloidosis.

For t-tau, our results suggest a fundamental shift in the interpretation of *GMNC* region. Our results suggest the association at this locus is notably stronger in females than in males, with a similar female-specific association observed in relation to age-of-onset in posthoc survival analyses. Further, our eQTL and gene expression results highlight comparable sex-specific effects in the associations between two genes in this locus and brain tau levels, suggesting the SNP association may be partially driven by *OSTN* or *CLDN16* rather than *GMNC*. *OSTN* expression levels are down-regulated in human neurons that are grafted into the brain of an AD mouse model [16], suggesting *OSTN* may be modulated by the presence of amyloidosis. It is also notable that *OSTN* is regulated by neuronal activity, but only in the primate brain, suggesting involvement in higher-order brain functions that are specific to primates [3]. *OSTN* is highly expressed in the neocortex of primates, and regulates dendritic growth [3]. Outside of the brain, the primary role of *OSTN* is in bone development, and *OSTN* responds to low-dose estradiol treatment *in vitro* [8], leaving open the possibility that *OSTN* function in brain may act in a hormone-dependent manner. Another candidate gene in this region was claudin 16 (*CLDN16*). *CLDN16* is a tight junction protein with a role in renal magnesium processing [27]. Other claudins have been implicated in AD previously [44, 49], and claudins 1, 11, and 16 are differentially expressed in the kidney of male compared to female rats [45]. Together with our results, these previous findings highlight *CLDN16* and *OSTN* as strong candidate genes that may have a sex-specific association with CSF t-tau.

One novel association locus on chromosome 4 was identified in amyloid analyses and showed a stronger association among females compared to males. The signal was proximal to a non-protein coding RNA *LINC00290,* but we did not observe any eQTL associations in the region. In gene expression analyses, we observed some evidence of a comparable female-specific association between gene expression and amyloidosis for one gene in this locus, teneurin transmembrane protein 3 (*TENM3*). *TENM3* is involved in neuronal development, axon guidance, and retinal mapping, and mutations in the gene cause the eye disorder microphthalmia [9]. *TENM3* has not been implicated in AD previously, but represents an interesting candidate gene for follow-up.

It is notable that the sex-specific associations observed in relation to amyloid and tau appeared to be driven by differences among the cognitively normal individuals in age-matched stratified analyses. However, the interpretation of results is challenging given the small sample size of the analyzed sub-groups. One possibility is that the observed sex-specific genetic effects are associated with deposition that occurs in the preclinical stage of disease, but larger datasets will be needed to fully explore the complex interplay between sex-specific genetic risk, age, and clinical status.

We also confirmed the sex difference in the association between *APOE* and CSF tau levels whereby females show a stronger association than males. We previously published this sex difference in a subset of the datasets here, and two additional datasets not included in this analysis (which lacked GWAS data) [26]. Across all the non-overlapping studies, the estimate of the sex interaction is strong. Only the Mayo dataset (see Table 1) showed an inverse direction of effect whereby the association between *APOE* and CSF total tau was slightly and non-significantly stronger in males compared to females. The Mayo dataset included the lowest percentages of females and *APOE* carriers and included older individuals on average, which may contribute to the divergent signal. Additional work is needed, particularly focusing on how selection criteria that emphasize memory impairment and a family history of AD could contribute to the observed sex difference in *APOE* associations with tau. Currently, the preponderance of data support a stronger association between *APOE* and tau among females.

The present study has multiple strengths, including the large sample size, clinical characterization in most datasets, inclusion of comprehensive eQTL analyses, and autopsy follow-up analyses that identified functional candidate genes that had sex-specific associations with AD neuropathology. Our results highlight the value of sex-specific analytical models for genetic association studies.

Our study has limitations. Our sample size did not provide adequate power to complete a full genome-wide interaction analysis that may highlight gene signals in opposite directions. The samples analyzed come from cohort studies that are highly educated and primarily White, limiting generalizability. Although we covaried for age and evaluated both age-matched and diagnosis stratified models, the sex differences in age and diagnosis are notable. A more carefully matched sample will be needed to better understand how sex differences in genetic risk for AD neuropathology change over the course of aging and disease. It is notable that the sex difference in the association between *APOE* and AD varies by age [41], suggesting that larger samples will be needed to better model genetic interactions with age on AD biomarker levels. Our gene expression results from ROS/MAP were measured from prefrontal cortex tissue, which was previously selected for measurement due to its relevance to human cognition and AD. However, RNA was not available from other brain regions for analysis, limiting our ability to evaluate differential associations across brain regions, particularly highly relevant regions like the hippocampus. Measurement in additional regions is ongoing, and future work delineating sex difference in gene expression associations across the brain are warranted.

In conclusion, our work has highlighted sex differences in the genetic predictors of AD biomarkers, including stronger associations between Serpin genes and amyloidosis among females. Modeling sex differences in GWAS analyses provides insights into novel genetic signals associated with disease, and provides a helpful framework for prioritizing and evaluating functional candidate genes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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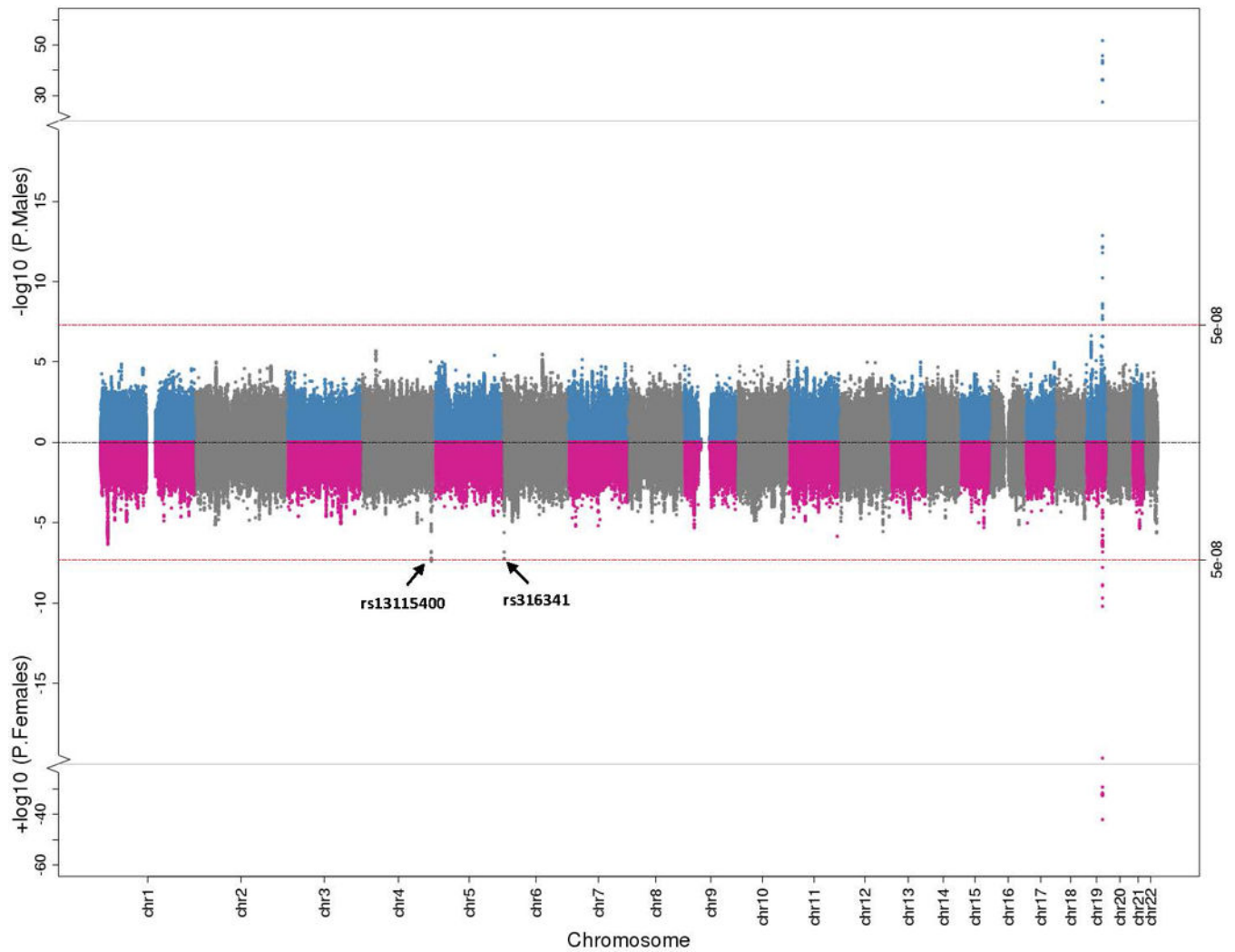
Fig. 1. Sex-Stratified Genome-Wide Association Results for CSF Aβ42
Miami plot illustrating CSF Aβ42 genome-wide association results stratified by males and females. Male findings are plotted in blue and grey on the top and female results are plotted in pink and grey at the bottom. The red lines at the top and bottom represent the genome-wide threshold for statistical significance ($p < 5 \times 10^{-8}$).
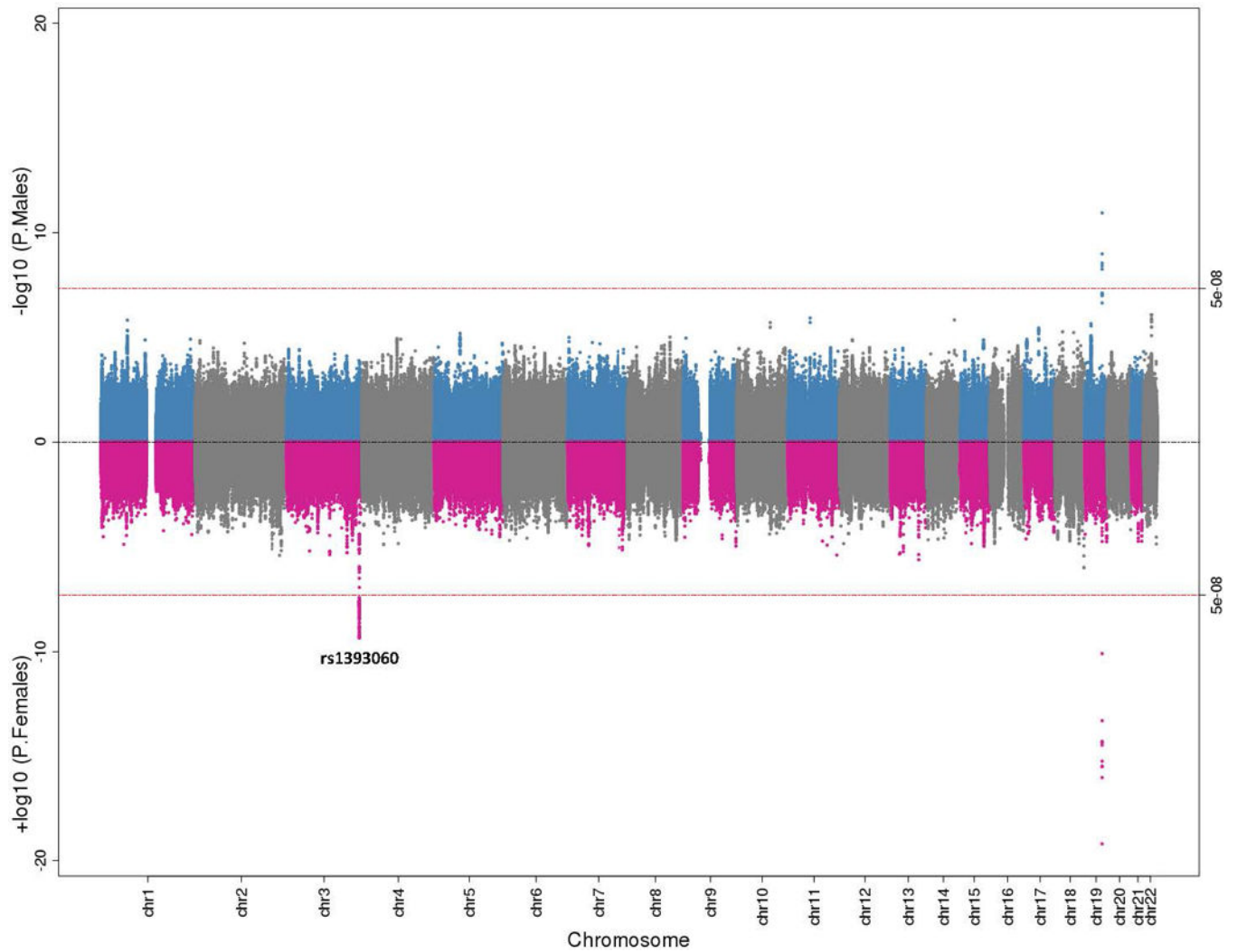
Fig. 2. Sex-Stratified Genome-Wide Association Results for CSF Tau

Miami plot illustrating CSF total tau genome-wide association results stratified by males and females. Male findings are plotted in blue and grey on the top and female results are plotted in pink and grey at the bottom. The red lines at the top and bottom represent the genome-wide threshold for statistical significance ($p < 5 \times 10^{-8}$).
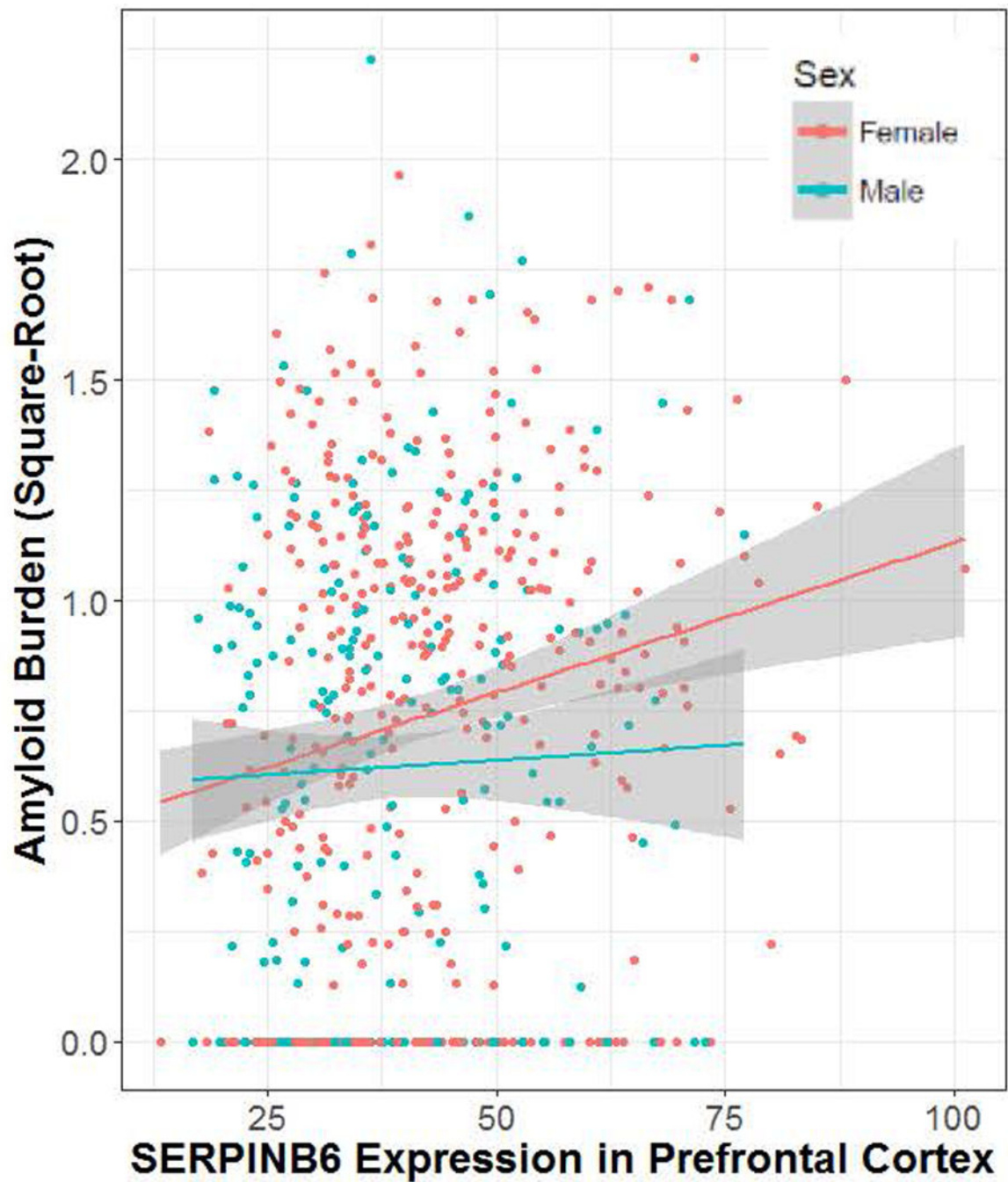
Fig. 3. Significant Sex-Specific Gene Expression Associations with Tangle Pathology Among Genes in the rs1393060 Locus

(a) *OSTN* and (b) *CLDN16* expression in the prefrontal cortex are presented on the X-axis, square-root transformed neurofibrillary tangle burden (measured with immunohistochemistry) is presented on the Y-axis. Females are presented in red and males are presented in blue.
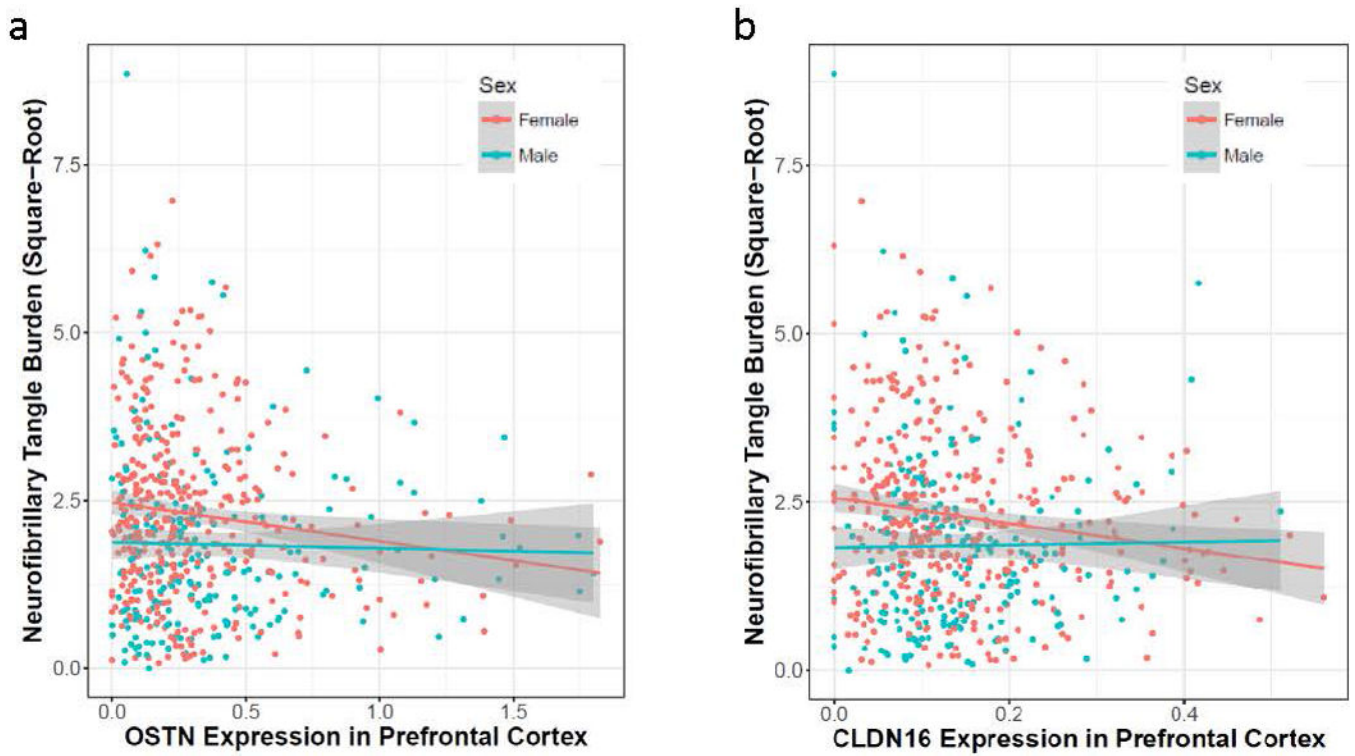
Fig. 4. Significant Sex-Specific Gene Expression Association Between *SERPINB6* Expression in the Prefrontal Cortex and Amyloid Burden

SERPINB6 expression in the prefrontal cortex are presented on the X-axis, square-root transformed amyloid burden (measured with immunohistochemistry) is presented on the Y-axis. Females are presented in red and males are presented in blue. Individuals with an amyloid burden value of 0 in the figure did not show IHC evidence of amyloidosis at autopsy.

Table 1.

Participant Characteristics

	Males	Females	P
Sample Size (n)	1527	1509	--
Age (years)	73.06 ± 10.66	70.85 ± 10.93	<0.001
% *APOE ε4*	43%	45%	0.15
% CDR > 0	51%	44%	<0.001
Normalized CSF Aβ42 (pg/mL) [*]	-0.03 ± 0.17	-0.02 ± 0.16	0.01
Normalized CSF Total Tau (pg/mL) [*]	-0.05 ± 0.23	-0.05 ± 0.24	0.67
Normalized CSF Phosphorylated Tau (pg/mL) [*]	-0.04 ± 0.20	-0.04 ± 0.21	0.82

[*] CSF levels of tau, ptau, and Aβ42 were log10-transformed and the mean from each data set was standardized to zero to account for the different platforms used by different studies to measure protein levels.

CDR=Clinical Dementia Rating

Table 2.

Sex-Specific Effects of Previously Reported Loci

SNP	Chr	Gene[+]	Function	Original β (SE)	Original P	Male β (SE)	Male P	Female β (SE)	Female P	Interaction β (SE)	Interaction P
CSF Aβ42											
rs185031519	1	*GLIS1*	Intergenic	**−0.06 (0.01)**	**2.08×10⁻⁸**	−0.06 (0.01)	1.06×10^{-4}	−0.06 (0.02)	7.92×10^{-5}	−0.003 (0.02)	0.90
rs316341	6	*SERPINB1*	Intronic	**−0.025 (0.004)**	**1.72×10⁻⁸**	−0.02 (0.01)	0.009	**−0.03 (0.01)**	**4.25×10⁻⁸**	−0.02 (0.01)	0.04 *
rs769449	19	*APOE*	Intronic	**−0.10 (0.005)**	**4.78×10⁻⁹⁴**	**−0.11 (0.01)**	**3.29×10⁻⁵³**	**−0.09 (0.01)**	**6.76×10⁻⁴³**	0.01 (0.01)	0.31
CSF Total Tau											
rs35055419	3	*GMNC*	Intergenic	**0.04 (0.01)**	**3.07×10⁻¹¹**	0.03 (0.01)	0.0003	**0.05 (0.01)**	**2.57×10⁻⁸**	0.02 (0.01)	0.17
rs769449	19	*APOE*	Intronic	**0.08 (0.01)**	**4.05×10⁻²⁹**	**0.07 (0.01)**	**4.41×10⁻¹²**	**0.09 (0.01)**	**5.58×10⁻²⁰**	0.02 (0.01)	0.10
CSF Phosphorylated Tau											
rs9527039	13	*PCDH8*	Intergenic	**−0.06 (0.01)**	**5.95×10⁻⁹**	−0.05 (0.01)	0.002	−0.08 (0.02)	5.64×10^{-7}	−0.03 (0.02)	0.15
rs12961169	18	*CTDP1*	Intergenic	**0.05 (0.01)**	**5.12×10⁻¹⁰**	0.05 (0.01)	1.16×10^{-5}	0.05 (0.01)	3.58×10^{-5}	−0.004 (0.02)	0.81
rs35055419	3	*GMNC*	Intergenic	**0.04 (0.01)**	**7.62×10⁻¹⁰**	0.03 (0.01)	0.0002	0.04 (0.01)	1.48×10^{-6}	0.01 (0.01)	0.45
rs514716	9	*GLIS3*	Intronic	**−0.05 (0.01)**	**2.94×10⁻⁸**	−0.04 (0.01)	0.0004	−0.05 (0.01)	3.45×10^{-5}	−0.01 (0.02)	0.51
rs769449	19	*APOE*	Intronic	**0.08 (0.01)**	**5.30×10⁻³³**	**0.07 (0.01)**	**5.83×10⁻¹⁴**	**0.09 (0.01)**	**4.31×10⁻²²**	0.02 (0.01)	0.10

Boldface font signifies genome-wide significant association

*
indicates interaction p<0.05.

[+]Gene listed is the closest gene and may not be the functional gene.

Table 3.

Genome-Wide Sex-Specific Effects

SNP	Chr	Gene[+]	Function	Male β (SE)	Male P	Female β (SE)	Female P	Interaction β (SE)	Interaction P
						CSF Aβ42			
rs13115400	4	*LINC00290*	Intergenic	0.01 (0.01)	0.20	**0.03 (0.01)**	3.97×10^{-8}	0.03 (0.01)	0.002 [*]
rs316341	6	*SERPINB1*	Intronic	−0.02 (0.01)	0.009	**−0.03 (0.01)**	4.25×10^{-8}	−0.02 (0.01)	0.04 [*]
SNP	Chr	Gene[+]	Function	Male β (SE)	Male P	Female β (SE)	Female P	Interaction β (SE)	Interaction P
						CSF Total Tau			
rs1393060	3	*GMNC*	Intergenic	0.02 (0.01)	0.03	**0.05 (0.01)**	4.57×10^{-10}	0.03 (0.01)	0.004 [*]

Boldface font signifies genome-wide significant association

[*] indicates interaction p<0.05.

[+] Gene listed is the closest gene and may not be the functional gene.

Table 4.

Replication of Sex-Specific Effects in Autopsy Samples from ROS/MAP

SNP	Chr	Gene[+]	Function	Amyloid Burden					
				Male β (SE)	Male P	Female β (SE)	Female P	Interaction β (SE)	Interaction P
rs316341	6	*SERPINB1*	Intronic	0.19 (0.10)	0.06	−0.001 (0.07)	0.99	−0.18 (0.12)	0.14
rs13115400	4	*LINC00290*	Intergenic	0.02 (0.09)	0.79	0.07 (0.06)	0.24	0.06 (0.11)	0.59

SNP	Chr	Gene[+]	Function	Neuronal Neurofibrillary Tangles					
				Male β (SE)	Male P	Female β (SE)	Female P	Interaction β (SE)	Interaction P
rs1393060	3	*GMNC*	Intergenic	−0.01 (0.10)	0.93	**0.14 (0.07)**	**0.047**	0.16 (0.12)	0.18

Boldface font signifies p<0.05.

[+] Gene listed is the closest gene and may not be the functional gene.