Sex modifies APOE ε4 dose effect on brain tau deposition in cognitively impaired individuals

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Abstract

Recent studies in cognitively unimpaired elderly individuals suggest that the APOE ε4 allele exerts a dosage-dependent effect on brain tau deposition. The aim of this study was to investigate sex differences in APOE ε4 gene dosage effects on brain tau deposition in cognitively impaired individuals using quantitative 18F-flortaucipir PET. Preprocessed 18F-flortaucipir tau PET images, T1-weighted structural MRI images, demographic information, global cortical amyloid-β burden measured by 18F-florbetapir PET, CSF total tau and phosphorylated tau measurements were obtained from the Alzheimer’s Disease Neuroimaging Initiative database. Two hundred and sixty-eight cognitively impaired individuals with 146 APOE ε4 non-carriers and 122 carriers (85 heterozygotes and 37 homozygotes) were included in the study. An iterative reblurred Van Cittert iteration partial volume correction method was applied to all downloaded PET images. MRI images were used for PET spatial normalization. Twelve regional standardized uptake value ratios relative to the cerebellum were computed in standard space. APOE ε4 dosage by sex interaction effect on 18F-flortaucipir standardized uptake value ratios was assessed using generalized linear models and sex-stratified analysis. We observed a significant APOE ε4 dosage by sex interaction effect on tau deposition in the lateral temporal, posterior cingulate, medial temporal, inferior temporal, entorhinal cortex,
amygdala, parahippocampal gyrus regions after adjusting for age and education level ($P < 0.05$). The medial temporal, entorhinal cortex, amygdala and parahippocampal gyrus regions retained a significant APOE $\varepsilon 4$ dosage by sex interaction effect on tau deposition after adjusting for global cortical amyloid-β ($P < 0.05$). In sex-stratified analysis, there was no significant difference in tau deposition between female homozygotes and heterozygotes ($P > 0.05$). In contrast, male homozygotes standardized uptake value ratios were significantly greater than heterozygotes or non-carriers throughout all twelve regions of interest ($P < 0.05$). Female heterozygotes exhibited significantly increased tau deposition compared to male heterozygotes in the orbitofrontal, posterior cingulate, lateral temporal, inferior temporal, entorhinal cortex, amygdala and parahippocampal gyrus ($P < 0.05$). Results from voxelwise analysis were similar to the ones obtained from regions of interest analysis. Our findings suggest that an APOE $\varepsilon 4$ dosage effect on brain region-specific tau deposition exists in males, but not females. These results have important clinical implications towards developing sex and genotype-guided therapeutics in Alzheimer’s disease and uncovers a potential explanation underlying differential apolipoprotein E $\varepsilon 4$-associated Alzheimer’s risk in males and females.

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**Abbreviations:** ADNI = Alzheimer’s Disease Neuroimaging Initiative; Aβ = amyloid-β; APOE = Apolipoprotein E; ROI = region of interest; SUVR = standardized uptake value ratio; MCI = mild cognitive impairment
Introduction

Alzheimer’s disease is the most common cause of dementia in elderly individuals. The strongest genetic modifier of late-onset Alzheimer’s disease is the Apolipoprotein E (APOE) ε4 allele.1 The APOE ε4 allele is associated with a heightened risk of developing Alzheimer’s disease, earlier age of onset and worse cognitive performance in a dose-dependent manner (i.e., the number of ε4 alleles in a person’s APOE genotype).2-4 In spite of these epidemiological and cognitive data, the dose effects of APOE ε4 on brain tau pathology remain unclear, especially in cognitively impaired cohorts.

18F-flortaucipir (also called 18F-T807 or 18F-AV-1451) is the first drug approved by Food and Drug Administration to image tau pathology in patients with Alzheimer’s disease. 18F-flortaucipir PET has been used to examine the effects of APOE ε4 in brain tau deposition. Analysis from genome-wide association studies, histopathology, CSF and tau PET imaging of the brain have consistently found a relationship between APOE ε4 and elevated tau pathology in cognitively unimpaired elderly individuals with mild cognitive impairment (MCI) and Alzheimer’s disease.5-10 Similarly, APOE ε4 carriers show accelerated brain amyloid-β (Aβ) accumulation relative to non-carriers. Furthermore, a recent study detected that the APOE ε4 allele is associated with increased entorhinal cortex tau standardized uptake value ratio (SUVR) in younger cognitively unimpaired individuals (47-70 years) in a genotype dosage-dependent manner.11 Similarly in another study involving patients with Alzheimer’s disease, APOE ε4 homozygotes had significantly more neurofibrillary tangles in the midfrontal, inferior parietal, superior temporal and hippocampus regions compared to either APOE ε4 heterozygotes or non-carriers.12 Another 18F-FDG PET study in patients with Alzheimer’s disease found that APOE
ε4 dosage is associated with glucose hypometabolism in the precuneus, posterior cingulate, parietotemporal, and frontal regions.\textsuperscript{13}

Previous studies have suggested that APOE ε4 confers a greater risk for Alzheimer’s disease, tau pathology, glucose hypometabolism and Aβ burden in females compared to males. Among APOE ε4 heterozygotes, the risk of developing Alzheimer’s disease for females is approximately 1.5 times greater than that of males.\textsuperscript{2} With regards to APOE ε4 and sex effects on tau pathology, CSF studies have demonstrated that APOE ε4 is more strongly associated with CSF tau in females compared with males.\textsuperscript{14-16} A PET study found that while APOE ε4 is associated with hypometabolism and greater Aβ burden across sex in MCI individuals, it is associated with greater Aβ burden only in males and not females among patients with Alzheimer’s disease.\textsuperscript{17} Several studies have investigated how sex modulates the effects of APOE ε4 on brain tau deposition measured by tau PET. A study in cognitively unimpaired subjects found that the association between CSF Aβ and tau accumulation measured by PET was strongest in female APOE ε4 carriers compared to other groups.\textsuperscript{18} Further, we have previously elucidated a sex by APOE ε4 carrier status interaction effect on tau deposition in the entorhinal cortex, amygdala, parahippocampal gyrus in individuals with MCI.\textsuperscript{5} Importantly, in all of these studies, the APOE ε4 genotype was analyzed as binary carrier/non-carrier variable, preventing the analysis of APOE ε4 dosage effects on brain tau deposition. A recent PET study has shown an APOE ε4 dosage effects on increased tau deposition in entorhinal cortex in younger cognitively unimpaired individuals (47-70 years).\textsuperscript{11} In light of overwhelming data supporting a sex by APOE ε4 carrier status interaction effect on tau pathology, sex by APOE ε4 dosage interaction effect on brain tau deposition should be explored.
The main aim of this study was to investigate sex differences in APOE ε4 dosage effect on brain tau deposition using data from the Alzheimer’s Disease Neuroimaging Initiative (ADNI). We hypothesized that APOE ε4 dosage effect on brain tau deposition are different in cognitively impaired females and males.

**Materials and methods**

**Participants**

Data in the study was obtained from the ADNI database. The ADNI study was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. Written informed consent was obtained from all individuals.

Two hundred and sixty-eight cognitively impaired individuals with either clinically diagnosed MCI (n = 197) or Alzheimer’s disease (n = 71) who underwent $^{18}$F-flortaucipir PET imaging in ADNI database were included in the study. Among the study participants, 188 subjects underwent $^{18}$F-florbetapir Aβ PET imaging and were used in subsequent analyses that controlled for global cortex Aβ burden. Global cortical SUVR values were downloaded from the ADNI-LONI database (adni.loni.usc.edu/methods/pet-analysis-method/pet-analysis/). Individuals with APOE ε2/ε4 allele, a putative protective allele for Alzheimer’s disease, were excluded. In total, 146 APOE ε4 non-carriers and 122 carriers (85 heterozygotes and 37 homozygotes) were included in the study. Performance on the Mini-Mental Status Examination (MMSE), Clinical Dementia Rating (CDR), Alzheimer's Disease Assessment Scale score (13 items; ADAS13), CSF total tau (t-tau), phosphorylated tau (p-tau) and Aβ42 levels were also obtained (Table 1). A full list of inclusion/exclusion criteria for ADNI study can be found at https://adni.loni.usc.edu/wp-content/uploads/2008/07/adni2-procedures-manual.pdf.

**APOE ε4 genotyping and gene dose**
Peripheral blood from study individuals was previously obtained by ADNI study investigators to be used for APOE ε4 genotyping. Restriction enzyme isoform genotyping was applied on DNA extracts to test for the presence of the APOE ε4 genotype. APOE ε4 dosage was defined as the number of APOE ε4 alleles (0, 1, or 2) carried by a participant.

**PET data acquisition and processing**

Raw T1-weighted structural MRI and preprocessed 18F-flortaucipir PET images were downloaded from the ADNI database (http://adni.loni.usc.edu/). The pre-processed PET images had been aligned, averaged, reoriented, interpolated into a standard space and smoothed with an 8 mm in full width at half maximum (FWHM) 3D Gaussian filter by the ADNI consortium. The details of tau PET acquisition parameters can be found at adni.loni.usc.edu/methods/pet-analysis-method/pet-analysis/. As described in our previous studies, we further processed the PET images with partial volume correction (PVC) and spatial normalization using Statistical Parametric Mapping (SPM12, Wellcome Department of Imaging Neuroscience, Institute of Neurology, London, UK) and MATLAB R2019b (The MathWorks Inc.). PVC was applied to the processed PET images to correct or minimize potential underestimation in PET measurement. In brief, an iterative reblurred Van Cittert method was used for PVC on the individual PET images, where a 3D Gaussian kernel of 8 mm FWHM was used for the spatial smoothing function with a step length $\alpha$ of 1.5. The iteration was stopped if the relative percent change of PVC images was < 1%. All the PET images were then coregistered to the individuals structural MRI images, which were normalized to standard Montreal Neurologic Institute (MNI) space using an MRI template (image volume: 121×145×121, voxel size: 1.5×1.5×1.5 mm in x, y, z). The transformation
parameters determined by MRI spatial normalization were then applied to the coregistered PET images for PET spatial normalization. SUVR images were calculated relative to the middle-inferior cerebellar gray matter reference region, which was drawn on 11 consecutive slices from $z = 27$ mm to $z = 17$ mm in the axial view from top of the head, as demonstrated in our previous studies.\textsuperscript{5, 24-26} For reference, SUVR images calculated from PET images without PVC were also analyzed.

A total of 12 cortical regions of interest (ROIs) were defined in the entorhinal cortex, parahippocampus, amygdala, inferior temporal, medial temporal, lateral temporal, posterior cingulate, posterior precuneus, parietal, orbitofrontal, superior frontal and prefrontal cortex.\textsuperscript{5, 21, 25} These ROIs were previously proposed by the Johns Hopkins Department of Radiology and manually drawn on the MRI template using PMOD (PMOD Technologies Ltd., Zürich, Switzerland) in the standard MNI space.\textsuperscript{21, 24, 26} To minimize variance resulting from the variability of ROI volume and shape in native space, the ROI SUVRs were calculated on the SUVR images in standard MNI space.\textsuperscript{5, 21, 27, 28}

**Statistical analysis**

All statistical analyses were performed using Statistical Analysis System (SAS version 9.4, SAS Institute, Inc) and SPM12. A generalized linear model was used to evaluate group differences among APOE $\varepsilon 4$ non-carriers, heterozygotes and homozygotes in age, MMSE, CDR, ADAS13 and CSF measurements, separately for females and males.

A generalized linear model was used to assess APOE $\varepsilon 4$ by sex interaction effects on global cortical $^{18}$F-florbetapir Aβ SUVR after controlling for age and years of education. This analysis revealed no significant APOE $\varepsilon 4$ by sex interaction effect ($P = 0.72$) or sex main effect ($P =$...
0.27) on global cortical $^{18}$F-florbetapir SUVR.

Two generalized linear models with and without controlling for global cortical $^{18}$F-florbetapir SUVR were fit to investigate APOE $\varepsilon$4 dosage by sex interaction effects on regional brain tau deposition as described earlier on the ROI level:

Model 1: ROI_SUVR ($^{18}$F-flortaucipir) $\sim$ Age + Educational level + Sex:APOE $\varepsilon$4 dosage

Model 2: ROI_SUVR ($^{18}$F-flortaucipir) $\sim$ Age + Educational level + Global cortex_SUVR ($^{18}$F-florbetapir) + Sex:APOE $\varepsilon$4 dosage

The APOE $\varepsilon$4 dosage by sex interaction effects on brain tau deposition was further evaluated by sex-stratified analysis. Specifically, group differences (i.e., APOE $\varepsilon$4 non-carriers, heterozygotes and homozygotes) in $^{18}$F-flortaucipir SUVR at the ROI and voxelwise levels were assessed for male and female, separately. In addition, we also evaluated differences in $^{18}$F-flortaucipir SUVR between males and females in APOE $\varepsilon$4 dosage groups. SAS was used for ROI analyses and SPM12 ($P < 0.001$, cluster size $> 100$ voxels) was used for voxelwise analysis as described previously. $^5,21,25$ The Benjamini-Hochberg method was used to control the false discovery rate (FDR) using the 12 study ROIs at both ROI-based and voxelwise levels. For ROI-based analyses, a FDR corrected $P$ value $< 0.05$ was defined as significant. For voxelwise based analyses, a FDR corrected value $P < 0.05$ and cluster size $> 100$ voxels was defined as significant.

**Data availability**

All data used in the current study were obtained from the ADNI database (available at https://adni.loni.usc.edu).
Results

Demographics

Two hundred and sixty-eight cognitively impaired individuals with 18F-flortaucipir PET imaging were included in our study. A full list of measures of demographic variables, Alzheimer’s disease cognition assessment, and measures of pathology including Aβ42 positivity, CSF Aβ42, CSF tau and CSF p-tau with statistical comparisons between APOE ε4 genotype groups is presented in Table 1. The overall APOE ε4 non-carrier, heterozygote and homozygote frequencies were 54%, 33% and 14%, respectively.

In female cohort, compared to non-carriers, APOE ε4 heterozygotes and homozygotes had decreased CSF Aβ42 (P < 0.01), increased CSF t-tau (heterozygotes: P < 0.01; homozygotes: P < 0.01) and increased p-tau (heterozygotes: P = < 0.01; homozygotes: P = < 0.01); APOE ε4 non-carriers, heterozygotes and homozygotes groups did not differ in MMSE score (P = 0.76), years of education (P = 0.55), CDR (P = 0.83) and ADAS13 (P = 0.20). In male cohort, compared to non-carriers, APOE ε4 homozygotes were younger (P = 0.02) and of lower MMSE (P = 0.02); APOE ε4 heterozygotes and homozygotes had decreased CSF Aβ42 levels (P < 0.01); APOE ε4 heterozygotes had increased t-tau (P = 0.04). We observed no significant differences among male non-carriers, heterozygotes and homozygotes in years of education (P = 0.88), CDR (P = 0.26), ADAS13 (P = 0.64), CSF t-tau (P = 0.21).

APOE ε4 dosage effect on regional 18F-flortaucipir SUVR in overall study cohort

We first investigated APOE ε4 dosage effects on regional tau deposition in the overall study cohort, including both males and females. There was a positive association between APOE ε4
dosage and ROI $^{18}$F-flortaucipir SUVRs in the prefrontal, superior frontal, parietal, posterior cingulate, posterior precuneus, medial temporal, and amygdala (Fig. 1).

**APOE ε4 dosage by sex interaction effect on regional $^{18}$F-flortaucipir SUVR**

ROIs with significant APOE ε4 dosage by sex interaction on regional $^{18}$F-flortaucipir SUVR were identified with and without controlling for global cortical $^{18}$F-florbetapir SUVR (Table 2) using the 12 ROIs described in the Methods. We observed a significant APOE ε4 dosage by sex interaction effect on $^{18}$F-flortaucipir tau deposition in the lateral temporal, posterior cingulate, medial temporal, inferior temporal, entorhinal cortex, amygdala, parahippocampal gyrus regions without adjusting for global cortical $^{18}$F-florbetapir SUVR (FDR $P \leq 0.05$; Table 2). The ROIs medial temporal, entorhinal cortex, amygdala and parahippocampal gyrus retained significant APOE ε4 dosage by sex interaction effect on tau deposition after adjusting for global cortical $^{18}$F-florbetapir SUVR (FDR $P < 0.05$; Table 2).

**Sex and APOE ε4 dosage-stratified analysis on regional $^{18}$F-flortaucipir SUVR**

The $^{18}$F-flortaucipir SUVR images with PVC (Fig. 2A) demonstrated increased contrast among APOE ε4 non-carriers, heterozygotes and homozygotes compared to the SUVR images without PVC (Fig. 2B) as reported previously in other cohorts.$^{5,21}$

The ROI-based SUVs of female APOE ε4 homozygotes were significantly higher than non-carriers in the medial temporal, entorhinal cortex, parahippocampus and amygdala after adjusting for age and years of education (FDR $P < 0.05$; Fig. 3). The ROIs of medial temporal, entorhinal cortex and amygdala retained a significant difference between homozygotes and
non-carriers after adjusting for global cortical $^{18}$F-florbetapir SUVR. Similarly, female heterozygotes had higher $^{18}$F-flortaucipir SUVR in the orbitofrontal, parietal, posterior cingulate, and posterior precuneus, lateral temporal, medial temporal, inferior temporal, entorhinal cortex, parahippocampus and amygdala (FDR $P < 0.05$; Fig. 3) compared to female non-carriers. The ROIs of medial temporal, entorhinal cortex, amygdala and parahippocampal gyrus retained a significant difference between heterozygotes and non-carriers after adjusting for global cortical $^{18}$F-florbetapir SUVR (FDR $P < 0.05$). Strikingly, there were no significant differences in any of the 12 ROIs SUVRs between female homozygotes and female heterozygotes with or without adjusting for global cortical $^{18}$F-florbetapir SUVR (FDR $P > 0.05$).

Among males, APOE ε4 homozygotes exhibited a marked increase in $^{18}$F-flortaucipir SUVR compared to both heterozygotes and non-carriers in all of the 12 ROIs. The entorhinal cortex, amygdala and parahippocampal gyrus retained a significant APOE ε4 dosage effect on tau deposition after adjusting for global cortical $^{18}$F-florbetapir SUVR. In addition, male heterozygotes had higher $^{18}$F-flortaucipir SUVR than male non-carriers in the medial temporal cortex and amygdala after adjusting for age and years of education (FDR $P < 0.05$; Fig. 3). No ROIs exhibit a significant difference between male heterozygotes and male non-carriers after adjusting for global cortical $^{18}$F-florbetapir SUVR (FDR $P > 0.05$).

Female heterozygotes exhibited significantly increased $^{18}$F-flortaucipir SUVR compared to male heterozygotes in the orbitofrontal, posterior cingulate, lateral temporal, inferior temporal, entorhinal cortex, amygdala and parahippocampal gyrus ($P < 0.05$; Fig. 3). There were no significant differences in ROIs $^{18}$F-flortaucipir SUVRs between males and females for both
homozygotes and non-carriers ($P > 0.05$).

**Sex-stratified effect of APOE ε4 dosage on voxelwise $^{18}$F-flortaucipir SUVR analysis**

Female APOE ε4 homozygotes had significantly higher $^{18}$F-flortaucipir SUVR than non-carriers in the clusters corresponding to the left middle temporal, inferior temporal, superior parietal; right middle frontal, superior frontal, bilateral parahippocampus, amygdala, entorhinal cortex, inferior parietal regions. Female heterozygotes had higher $^{18}$F-flortaucipir SUVR than non-carriers in an even greater number of clusters involving the temporal cortex, middle cingulate, and precuneus locations (FDR $P < 0.05$). No significant differences between female homozygotes and heterozygotes were found (Fig. 4A and Table 3).

Male APOE ε4 homozygotes showed significantly higher $^{18}$F-flortaucipir SUVR than non-carriers in the bilateral middle temporal, inferior temporal, parahippocampus, amygdala, fusiform, entorhinal cortex, middle cingulate, precuneus, inferior parietal, middle frontal, superior frontal, and left superior parietal regions. Furthermore, male homozygotes showed higher $^{18}$F-flortaucipir SUVR than heterozygotes in right middle temporal, inferior temporal, precuneus, bilateral middle cingulate, and inferior parietal locations (FDR $P < 0.05$). No significant differences in $^{18}$F-flortaucipir SUVR between male heterozygotes and male non-carriers were found (Fig. 4B and Table 3). Further, there were no cerebral locations where SUVR was higher in the male non-carriers compared to either the male heterozygotes or male homozygotes.

**Discussion**

The main finding from this study is that females and males show different patterns of APOE
ε4 dosage-related tau deposition in cognitively impaired elderly individuals. Specifically, in females, increased tau deposition was observed in both APOE ε4 heterozygotes and homozygotes compared to non-carriers. But, in males, only the APOE ε4 homozygotes (and not the heterozygotes) had increased tau compared to non-carriers. Together, these results suggest that only one APOE ε4 allele is sufficient to increase tau accumulation in females, while two APOE ε4 alleles are needed to cause a similar effect in males. Consistent with previous studies,29, 30 we found that ROIs of regions entorhinal cortex, amygdala and parahippocampal gyrus showed an Aβ-independent association between the APOE ε4 gene dosage and tau deposition in males but not females after controlling for global cortex 18F-florbetapir SUVR. These findings have important clinical implications towards developing sex and genotype-guided therapeutics in Alzheimer’s disease and uncovers a possible explanation underlying differential apolipoprotein E ε4-associated Alzheimer’s risk in males and females.

Our findings suggest that the three levels of APOE ε4 alleles exert different effects on tau deposition in males and females. In males, a significant APOE ε4 dose effect on tau deposition was observed throughout the cortex, especially in medial temporal and amygdala (homozygotes > heterozygotes > non-carriers), but no APOE ε4 dosage effect was observed among females. These results were consistent across ROI-based and voxelwise analyses. Our findings are similar to the recent 18F-flortaucipir PET study which demonstrated an APOE ε4 dose-dependent effect on entorhinal cortex tau deposition in cognitively unimpaired individuals.11 The current findings are in line with our group’s previous results that female APOE ε4 carriers had greater tau deposition than males in the entorhinal cortex, amygdala and parahippocampal gyrus.5 Our findings are also in line with the prior studies, which showed that
APOE ε4 heterozygosity is sufficient to increase Alzheimer’s disease risk in females, while in males, APOE ε4 homozygosity is required to increase Alzheimer’s disease risk.\textsuperscript{1, 2, 31, 32} Taken together, our data suggest that females may have higher susceptibility to the APOE ε4 allele than males. These findings also consistent with work from Payami et al.\textsuperscript{32} showing that the age of Alzheimer’s disease onset among female APOE ε4 heterozygotes was similar to homozygotes, but younger than non-carriers. In contrast, in males, the age of Alzheimer’s disease onset among APOE ε4 heterozygotes was similar to non-carriers, but younger than homozygotes.\textsuperscript{32} Another study suggested that females have a higher risk of Alzheimer’s disease than males not because of their greater longevity,\textsuperscript{33} but likely due to the female-specific susceptibility among heterozygotes.\textsuperscript{2, 32} Combined with these previous findings and results for the study, provide a potential genetic-informed explanation underlying increased susceptibility to Alzheimer’s disease in females.\textsuperscript{34}

Alzheimer’s disease pathology is characterized by the accumulation of Aβ plaques and neurofibrillary tangles in the brain. Our study found an Aβ-independent association between APOE ε4 dosage and tau deposition only in regions of early tau deposition (medial temporal, entorhinal cortex, amygdala and parahippocampal gyrus). This suggests that APOE ε4 dosage by sex interaction effect on tau deposition were partially mediated by Aβ. In line with this, a biochemical study found that accumulations of tau and Aβ occur independently in the entorhinal cortex.\textsuperscript{35} Two recent cross-sectional tau PET studies indicated that APOE ε4 is associated with tau deposition in the medial temporal cortex independent of Aβ status.\textsuperscript{30, 36} The neocortex Aβ-dependent tau deposition maybe explained by the dual-cascade hypothesis.\textsuperscript{37} However, our results are in contrast to a recent longitudinal tau PET study, which showed that
the accumulation rate of tau in temporal meta- and neocortical ROIs is increased in females Aβ-positive individuals.\textsuperscript{38} It should be noted that the analyses with controlling for global cortical \textsuperscript{18}F-florbetapir SUVR were performed in a subset of the cohort of 188 individuals. Therefore, the reduced ROIs of APOE ε4 dosage by sex interaction effect after controlling for \textsuperscript{18}F-florbetapir SUVR may be partly explained by differences in sample size. We realized that the time interval between the \textsuperscript{18}F-florbetapir and \textsuperscript{18}F-flortaucipir PET scans was 4.35 months (SD: 5.68; range: 0-27.53 months), which were unlikely to be a source of error as confirmed by analyses with and without controlling the interval of the two scans.

Past report has demonstrated that the effect of APOE ε4 on CSF t-tau and p-tau levels is stronger in females than males.\textsuperscript{14} Sundermann et al.\textsuperscript{17} showed that APOE ε4 is associated with greater Aβ burden across males and females in MCI individuals, but only in males for Alzheimer’s disease individuals. These findings are in line with the previous work of our group and others showing that both t-tau and p-tau increased more in female APOE ε4 carriers compared to males.\textsuperscript{5, 16} This lack of concordance of APOE ε4 dose effects between brain \textsuperscript{18}F-flortaucipir SUVR and CSF tau is probably due to the limited number of samples with CSF information, reducing statistical power in CSF analysis. In the present study, the interval between tau PET and CSF biomarkers was 21.07 months (SD: 30.29; range: 0-128 months) which may reduce the statistical power on the analysis of the APOE ε4 dose effect on CSF t-tau and p-tau. The inconsistency between tau PET and CSF tau may be due to the increased tau in CSF reflects not regional tau deposition, but neuronal damage and disease progression. Compared to CSF tau assessments, brain tau PET provides quantitative brain tau measurements with higher sensitivity and specificity in Alzheimer’s disease study.\textsuperscript{39}
Nevertheless, there were some limitations to this study. Even in our large study cohort of 268 cognitively impaired participants, only 37 individuals were homozygous for the APOE ε4 allele. This limits our ability to accurately model the effects of APOE ε4 dosage across the spectrum of Alzheimer’s disease severity and age ranges. As reported in a prior study, APOE ε4 homozygotes may have an increased risk of Alzheimer’s disease before their 70s, but resilience to Alzheimer’s disease beyond their 70s.\textsuperscript{11} Further, our study involved a relatively small number of APOE ε4 carriers (69 male APOE ε4 carriers and 53 female APOE ε4 carriers), potentially limiting the generalizability of our results, although a generalized linear model to assess group differences in tau deposition was used for its robustness with smaller sample sizes. The statistical power for \textsuperscript{18}F-flortaucipir SUVR to distinguish between APOE ε4 heterozygotes and homozygotes ranged from 0.97 to 0.999 in all 12 ROIs among males, and 0.05 to 0.69 among females. The results of this study will be evaluated further in the near future on the ongoing ADNI projects and other larger cohort studies.

In conclusion, this quantitative \textsuperscript{18}F-flortaucipir PET study in individuals with cognitive impairment showed that an APOE ε4 gene dose-dependent effect on brain region-specific tau deposition exists in males, but not in females. This work highlights the importance of considering sex and APOE ε4 dose effect in biomarker development and mechanistic studies in Alzheimer’s disease using quantitative \textsuperscript{18}F-flortaucipir PET.

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Competing interests

The authors report no competing interests.

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Figure legends

Figure 1 APOE ε4 dose effect on ROI 18F-flortaucipir SUVRs in the study cohort of cognitively impaired individuals. Mean (±standard deviation) of SUVR for APOE ε4 non-carriers (gray bar), heterozygotes (blue bar) and homozygotes (pink bar) are depicted. P value was defined using a generalized linear model, adjusting for age, years of education and sex. SUVR: Standardized uptake value ratio. * P < 0.05, ** P < 0.01, *** P < 0.001.

Figure 2 Mean images with and without PVC illustrate that sex modulates the APOE ε4 dose effect on 18F-flortaucipir SUVR in cognitively impaired participants. Partial volume corrected images (A) show increased contrast and spatial resolution compared to images without PVC (B). Both PVC and non-PVC images visually demonstrate an interaction effect between sex and APOE ε4 status. PVC: partial volume correction, SUVR: standardized uptake value ratio.

Figure 3 Sex modifies the APOE ε4 dosage effect on ROI-based analysis of 18F-flortaucipir PET in cognitively impaired individuals. Box plots depict median value and the interquartile ranges of regional SUVRs for APOE ε4 non-carriers (green), heterozygotes (purple) and homozygotes (red) in females and males. P value was defined using a generalized linear model, adjusting for age and years of education. F: female, M: male, SUVR: Standardized uptake value ratio. Bold significance lines indicate comparison between APOE ε4 dosage groups. * P < 0.05, ** P < 0.01, *** P < 0.001.

Figure 4 Sex modifies the APOE ε4 dose effect on voxelwise analysis of 18F-flortaucipir PET in cognitively impaired participants. APOE ε4 dose effect on voxelwise SUVR is depicted in females (A) and males (B). Female heterozygotes (A, left) and homozygotes (A,
right) display increased $^{18}$F-flortaucipir SUVR compared to non-carriers. No significant differences in SUVR were observed between female heterozygotes and homozygotes. Male homozygotes demonstrate increased $^{18}$F-flortaucipir SUVR compared to male heterozygotes (B, left) and male non-carriers (B, right). No significant differences in SUVR were observed between male heterozygotes and male non-carriers. T values are expressed on blue-red scale from 0 to 5 depicting voxels level with $P < 0.001$ (adjusted for age and years of education). SUVR: standardized uptake value ratio.
Figure 1

195x145mm (300 x 300 DPI)
Figure 2

37x47mm (300 x 300 DPI)
Figure 3

196x151mm (300 x 300 DPI)
Figure 4

APOE ε4 dose effect on \(^{18}\text{F}\)-flortaucipir PET SUVR in females

APOE ε4 homoyzogotes > non-carriers

APOE ε4 heterozygotes > non-carriers

left right

APOE ε4 dose effect on \(^{18}\text{F}\)-flortaucipir PET SUVR in males

APOE ε4 homoyzogotes > non-carriers

APOE ε4 homoyzogotes > heterozygotes

left right

65x61mm (300 x 300 DPI)
### Table 1 Clinical characteristics of the study cohort

| Parameter (±SD) | Female | | | Male | | |
|----------------|--------|--------|--------|--------|--------|--------|
| | Non-carriers (n=54) | Heterozygotes (APOE ε3 ε4) (n=38) | Homozygotes (APOE ε4 ε4) (n=15) | Non-carriers (n=92) | Heterozygotes (APOE ε3 ε4) (n=47) | Homozygotes (APOE ε4 ε4) (n=22) |
| Alzheimer’s disease/ MCI, No. | 12/42 | 11/27 | 5/10 | 17/75 | 17/30 | 9/13 |
| Age, years ± SD | 76.62 ± 9.23 (56–93) | 73.70 ± 7.26 (61–88) | 69.86 ± 6.79 (57–82) | 78.13 ± 8.06 (57–94) | 77.49 ± 7.57 (59–90) | 73.38 ± 9.77 (56–91) |
| Education, years ± SD | 15.19 ± 2.37 | 14.89 ± 2.22 | 15.67 ± 2.58 | 16.61 ± 2.66 | 16.51 ± 2.76 | 16.86 ± 3.17 |
| MMSE, score ± SD | 26.17 ± 4.55 | 25.50 ± 3.94 | 25.67 ± 4.53 | 27.04 ± 3.60 | 25.81 ± 3.88 | 25.00 ± 3.83* |
| CDR, score ± SD | 0.62 ± 0.49 | 0.63 ± 0.40 | 0.70 ± 0.41 | 0.53 ± 0.28 | 0.60 ± 0.46 | 0.66 ± 0.36 |
| ADAS13, score ± SD | 21.52 ± 11.11 | 17.59 ± 9.51 | 19.38 ± 7.62 | 18.09 ± 8.21 | 19.29 ± 8.66 | 17.30 ± 11.86 |
| 18F-florbetapir PET, No. | 44 | 23 | 9 | 66 | 30 | 16 |
| Aβ positive (%) | 25/54 (46.30%) | 32/38 (84.21%) | | 15/15 (100.00%) | 35/92 (32.61%) | 37/47 (78.72%) |
| APOE ε2 ε4, No. | 5 | | | | | |
| CSF Aβ42 (pg/mL ± SD) | 1379.30 ± 728.11 | 845.80 ± 459.71* | 613.70 ± 217.87* | 1314.27 ± 763.47 | 786.86 ± 426.06* | 499.36 ± 124.55* |
| CSF t-tau (pg/mL ± SD) | 265.65 ± 128.30 | 370.47 ± 148.29* | 406.11 ± 250.91* | 261.42 ± 121.17 | 261.42 ± 121.17 | 280.17 ± 97.14 |
| CSF p-tau (pg/mL ± SD) | 23.99 ± 13.78 | 37.06 ± 18.71* | 43.18 ± 30.94* | 24.22 ± 13.37 | 30.06 ± 12.07* | 28.19 ± 11.07 |

Notes: a: \( P < 0.05 \) compared to non-carriers, b: \( P < 0.01 \) compared to non-carriers; c: \( P < 0.05 \) compared to heterozygotes, d: \( P < 0.01 \); ADAS: Alzheimer’s Disease Assessment Scale; Aβ: Amyloid-β; CDR: Clinical Dementia Rating Scale; CSF: cerebrospinal fluid, MMSE: Mini-Mental State Examination. Aβ status was positive (negative) if Aβ load was higher (lower) than 18F-florbetaben cortical SUVR based on the whole cerebellum reference =1.08 and 18F-florbetapir =1.11 (adni.loni.usc.edu/methods).
Table 2 APOE ε4 dosage by sex interaction effect on tau deposition in cognitively impaired participants

| ROIs                  | Not adjusted for global cortical amyloid level | Adjusted for global cortical amyloid level |
|-----------------------|-----------------------------------------------|------------------------------------------|
|                       | Standardized β (95%CI) | APOE ε4 × sex | FDR | Standardized β (95%CI) | APOE ε4 × sex | FDR |
| Orbital Frontal       | −0.08 (−0.13 to 0.05) | 0.04 | 0.06 | −0.02 (−0.11 to 0.10) | 0.82 | 0.95 |
| Prefrontal            | −0.07 (−0.13 to 0.06) | 0.12 | 0.14 | −0.01 (−0.11 to 0.09) | 0.88 | 0.95 |
| Superior Frontal      | −0.03 (−0.13 to 0.09) | 0.38 | 0.38 | −0.01 (−0.13 to 0.12) | 0.80 | 0.95 |
| Lateral Temporal      | −0.03 (−0.12 to 0.18) | 0.02 | 0.04 | 0.09 (−0.09 to 0.25) | 0.20 | 0.43 |
| Parietal              | 0.00 (−0.14 to 0.14) | 0.18 | 0.20 | 0.03 (−0.13 to 0.18) | 0.57 | 0.93 |
| Posterior Precuneus   | −0.06 (−0.20 to 0.09) | 0.05 | 0.07 | −0.03 (−0.18 to 0.14) | 0.96 | 0.96 |
| Posterior Cingulate   | 0.01 (−0.16 to 0.18) | 0.02 | 0.04 | 0.06 (−0.13 to 0.25) | 0.30 | 0.56 |
| Medial Temporal       | 0.15 (−0.01 to 0.21) | 0.002 | 0.01 | 0.22 (0.04 to 0.28) | 0.006 | 0.03 |
| Inferior Temporal     | 0.03 (−0.14 to 0.19) | 0.03 | 0.05 | 0.10 (−0.08 to 0.29) | 0.16 | 0.42 |
| Entorhinal Cortex     | 0.21 (0.03 to 0.33) | <0.001 | <0.001 | 0.30 (0.12 to 0.47) | <0.001 | 0.001 |
| Amygdala              | 0.12 (−0.04 to 0.26) | 0.008 | 0.03 | 0.19 (0.02 to 0.37) | 0.02 | 0.07 |
| Parahippocampal       | 0.16 (−0.01 to 0.21) | <0.001 | <0.001 | 0.23 (0.04 to 0.29) | 0.002 | 0.01 |

Notes: 95% CI represents the 95% confidence interval of the APOE ε4 dosage by sex coefficient.

*P* value was defined using a generalized linear model to detect significant APOE ε4 dosage by sex interaction effect in cognitively impaired individuals. Age and education were included as covariates. Global cortical amyloid SUVR was also included as a covariate in the right column using 188 individuals with 18F-florbetapir PET data. FDR *P* value was defined using Benjamini-Hochberg procedure to control the false discovery rate.

APOE: apolipoprotein E; FDR: false discovery rate.
Table 3 Clusters with significant association between $^{18}$F-flortaucipir SUVR and APOE ε4 dosage

| Clusters                  | Female                      | Male                        |
|---------------------------|-----------------------------|-----------------------------|
|                           | Female Non-carriers >       | Male Heterozygotes >        |
|                           | Male Non-carriers >         | Male Male Non-carriers >    |
|                           | Homozygotes >               | Homozygotes >               |
|                           | Heterozygotes >             | Heterozygotes >             |
|                           | x' y' z'                    | x' y' z'                    |
| Temporal_Mid_L            | −63 −28.5 −21              | −34 −26 −5                 |
| Temporal_Inf_L            | −36 −21 −6                 | −25 −16 −3                |
| Temporal_Mid_R            | 55                       | 58 −21 −7                 |
| Temporal_Inf_R            | −96 −33 −16.5             | 57 −6 −2                  |
| Temporal_Sup_R            | −18 −9 −18                | −18 −4 −22                |
| Amygdala_L                | −22 −12 −25               | −21 −6 −21                |
| ParaHippocampal_L         | 64.5 −33 −16.5            | 57 −6 −2                  |
| Amygdala_R                | −18 −9 −18                | −18 −4 −22                |
| Cingulum_Mid_L            | −27 −7.5 −18              | 36 −6 −22 −36             |
| Precuneus_L               | −6 −43 −21                | −24 −41                  |
| Precuneus_R               | 9 −43 −21                 | 26 −24 −41                |
| Cingulum_Mid_R            | −52.5 −52.5 −42           | −42 −48 −48               |
| Precuneus_L               | −44 −48 −40               | −42 −48 −43               |
| Parietal_Inf_L            | 34.5 −52.5 −46.5          | −45 −53 −53               |
| Parietal_Inf_R            | −31.5 −63 −60             | −33 −54 −54               |
| Parietal_Sup_L            | −30 −67.5 −57             | −31 −54 −54               |
| Frontal_Mid_L             | −4 −59 −17                | −4 −59 −17                |
| Frontal_Sup_L             | 13.5 −27 −57              | 13.5 −27 −57              |
| Frontal_Mid_R             | 28.5 −34.5 −36            | 28.5 −34.5 −36            |
| Frontal_Sup_R             | −36 −21 −6                | −36 −21 −6                |

Notes: Data extracted from SPM12 analysis showing voxels with significantly increased $^{18}$F-Flortaucipir SUVR in cognitively impaired female APOE ε4 homozygotes versus non-carriers, female heterozygotes versus female non-carriers, male APOE ε4 homozygotes versus male non-carriers, male homozygotes versus male heterozygotes, adjusted for age and years of education. There were no significant differences between female homozygotes and female heterozygotes or between male heterozygotes and male non-carriers (not listed in Table 3). Cluster locations correspond to the brain maps shown in Figure 3. Atlas coordinates were obtained from Automated Anatomical Labeling (AAL). 40