





Sex modifies APOE ϵ 4 dose effect on brain tau deposition in cognitively impaired individuals

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Recent studies in cognitively unimpaired elderly individuals suggest that the APOE ϵ 4 allele exerts a dosage-dependent effect on brain tau deposition. The aim of this study was to investigate sex differences in APOE ϵ 4 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, 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 ϵ 4 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. Magnetic resonance images were used for PET spatial normalization. Twelve regional standardized uptake value ratios relative to the cerebellum were computed in standard space. APOE ϵ 4 dosage \times 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 ϵ 4 dosage \times 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 ϵ 4 dosage \times 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 12 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 voxel-wise analysis were similar to the ones obtained from regions of interest analysis.

Our findings indicate that an APOE ϵ 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 APOE ϵ 4-associated Alzheimer's risk in males and females.

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Abbreviations: ADNI = Alzheimer's Disease Neuroimaging Initiative; PVC = partial volume correction; SUVR = standardized uptake value ratio

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) $\epsilon 4$ allele.¹ The APOE $\epsilon 4$ 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 $\epsilon 4$ alleles in a person's APOE genotype).^{2–4} In spite of these epidemiological and cognitive data, the dose effects of APOE $\epsilon 4$ on brain tau pathology remain unclear, especially in cognitively impaired cohorts.

^{18}F -flortaucipir (also called ^{18}F -T807 or ^{18}F -AV-1451) is the first drug approved by the Food and Drug Administration to image tau pathology in patients with Alzheimer's disease. ^{18}F -flortaucipir PET has been used to examine the effects of APOE $\epsilon 4$ 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 $\epsilon 4$ and elevated tau pathology in cognitively unimpaired elderly individuals with mild cognitive impairment and Alzheimer's disease.^{5–10} Similarly, APOE $\epsilon 4$ carriers show accelerated brain amyloid- β accumulation relative to non-carriers. Furthermore, a recent study detected that the APOE $\epsilon 4$ 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 $\epsilon 4$ homozygotes had significantly more neurofibrillary tangles in the midfrontal, inferior parietal, superior temporal and hippocampus regions compared to either APOE $\epsilon 4$ heterozygotes or non-carriers.¹² Another ^{18}F -FDG PET study in patients with Alzheimer's disease found that APOE $\epsilon 4$ dosage is associated with glucose hypometabolism in the precuneus, posterior cingulate, parietotemporal and frontal regions.¹³

Previous studies have suggested that APOE $\epsilon 4$ confers a greater risk for Alzheimer's disease, tau pathology, glucose hypometabolism and amyloid- β burden in females compared to males. Among APOE $\epsilon 4$ heterozygotes, the risk of developing Alzheimer's disease for females is approximately 1.5 times greater than that of males.² In regard to APOE $\epsilon 4$ and sex effects on tau pathology, CSF studies have demonstrated that APOE $\epsilon 4$ is more strongly associated with CSF tau in females compared with males.^{14–16} A PET study found that while APOE $\epsilon 4$ is associated with hypometabolism and greater amyloid- β

burden across sex in individuals with mild cognitive impairment, it is associated with greater amyloid- β burden only in males and not females among patients with Alzheimer's disease.¹⁷ Several studies have investigated how sex modulates the effects of APOE $\epsilon 4$ on brain tau deposition measured by tau PET. A study in cognitively unimpaired participants found that the association between CSF amyloid- β and tau accumulation measured by PET was strongest in female APOE $\epsilon 4$ carriers compared to other groups.¹⁸ Further, we have previously elucidated a sex \times APOE $\epsilon 4$ carrier status interaction effect on tau deposition in the entorhinal cortex, amygdala, parahippocampal gyrus in individuals with mild cognitive impairment.⁵ Importantly, in all of these studies, the APOE $\epsilon 4$ genotype was analysed as binary carrier/non-carrier variable, preventing the analysis of APOE $\epsilon 4$ dosage effects on brain tau deposition. A recent PET study has shown an APOE $\epsilon 4$ dosage effect on increased tau deposition in the entorhinal cortex in younger cognitively unimpaired individuals (47–70 years).¹¹ In light of overwhelming data supporting a sex \times APOE $\epsilon 4$ carrier status interaction effect on tau pathology, a sex \times APOE $\epsilon 4$ dosage interaction effect on brain tau deposition should be explored.

The main aim of this study was to investigate sex differences in APOE $\epsilon 4$ dosage effect on brain tau deposition using data from the Alzheimer's Disease Neuroimaging Initiative (ADNI). We hypothesized that APOE $\epsilon 4$ dosage effect on brain tau deposition are different in cognitively impaired females and males.

Materials and methods

Participants

Data in the study were 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.

From the ADNI database, 268 cognitively impaired individuals with either clinically diagnosed mild cognitive impairment ($n = 197$) or Alzheimer's disease ($n = 71$) who underwent ^{18}F -flortaucipir PET imaging were included in the study. Among the study participants, 188 individuals underwent ^{18}F -florbetapir amyloid- β PET imaging and were used in subsequent analyses that controlled for global cortex amyloid- β burden. Global cortical SUVR values were downloaded from the ADNI-LONI database (adni.loni.usc.edu/methods/pet-analysis-method/pet-analysis/). Individuals with the APOE $\epsilon 2/\epsilon 4$ allele, a putative protective allele for

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, n	12/42	11/27	5/10	17/75	17/30	9/13
Age, years ± SD (range)	76.62 ± 9.23 (56–93)	73.70 ± 7.26 (61–88)	69.86 ± 6.75 ^b (57–82)	78.13 ± 8.06 (57–94)	77.49 ± 7.57 (59–90)	73.38 ± 9.77 ^a (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 ^a
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
¹⁸ F-florbetapir PET, n	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)	21/22 (95.45)
APOE ε2 ε4, n		5			5	
CSF	n = 43	n = 29	n = 13	n = 71	n = 30	n = 19
CSF Aβ ₄₂ (pg/ ml ± SD)	1379.30 ± 728.11	845.80 ± 459.71 ^b	613.70 ± 217.87 ^b	1314.27 ± 763.47	786.86 ± 426.06 ^b	499.36 ± 124.55 ^b
CSF t-tau (pg/ ml ± SD)	265.65 ± 128.30	370.47 ± 148.29 ^b	406.11 ± 250.91 ^b	261.42 ± 121.17	305.03 ± 105.58	280.17 ± 97.14
CSF p-tau (pg/ ml ± SD)	23.99 ± 13.78	37.06 ± 18.71 ^b	43.18 ± 30.94 ^b	24.22 ± 13.37	30.06 ± 12.07 ^a	28.19 ± 11.07

ADAS = Alzheimer's Disease Assessment Scale; Aβ = amyloid-β; CDR = Clinical Dementia Rating Scale; MMSE = Mini-Mental State Examination. Amyloid-β status was positive (negative) if amyloid-β 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).

^aP < 0.05 compared to non-carriers.

^bP < 0.01 compared to non-carriers.

Alzheimer's disease, were excluded.¹⁹ 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 amyloid-β₄₂ 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.²⁰ 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 preprocessed 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,^{5,21,22} 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 α of 1.5. The iteration was stopped if the relative percentage change of PVC images was < 1%.²³ 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 co-registered PET images for PET spatial normalization. SUVR images were calculated relative to the middle-inferior cerebellar grey matter reference region, which was drawn on 11 consecutive slices from z = 27 to z = 17 mm in the axial view from top of the head, as demonstrated in our previous studies.^{5,24–26} For reference, SUVR images calculated from PET images without PVC were also analysed.

A total of 12 cortical regions of interest 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.^{5,21,25} These region of interest 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.^{21,24,26} To minimize variance resulting from the variability of region of interest volume and

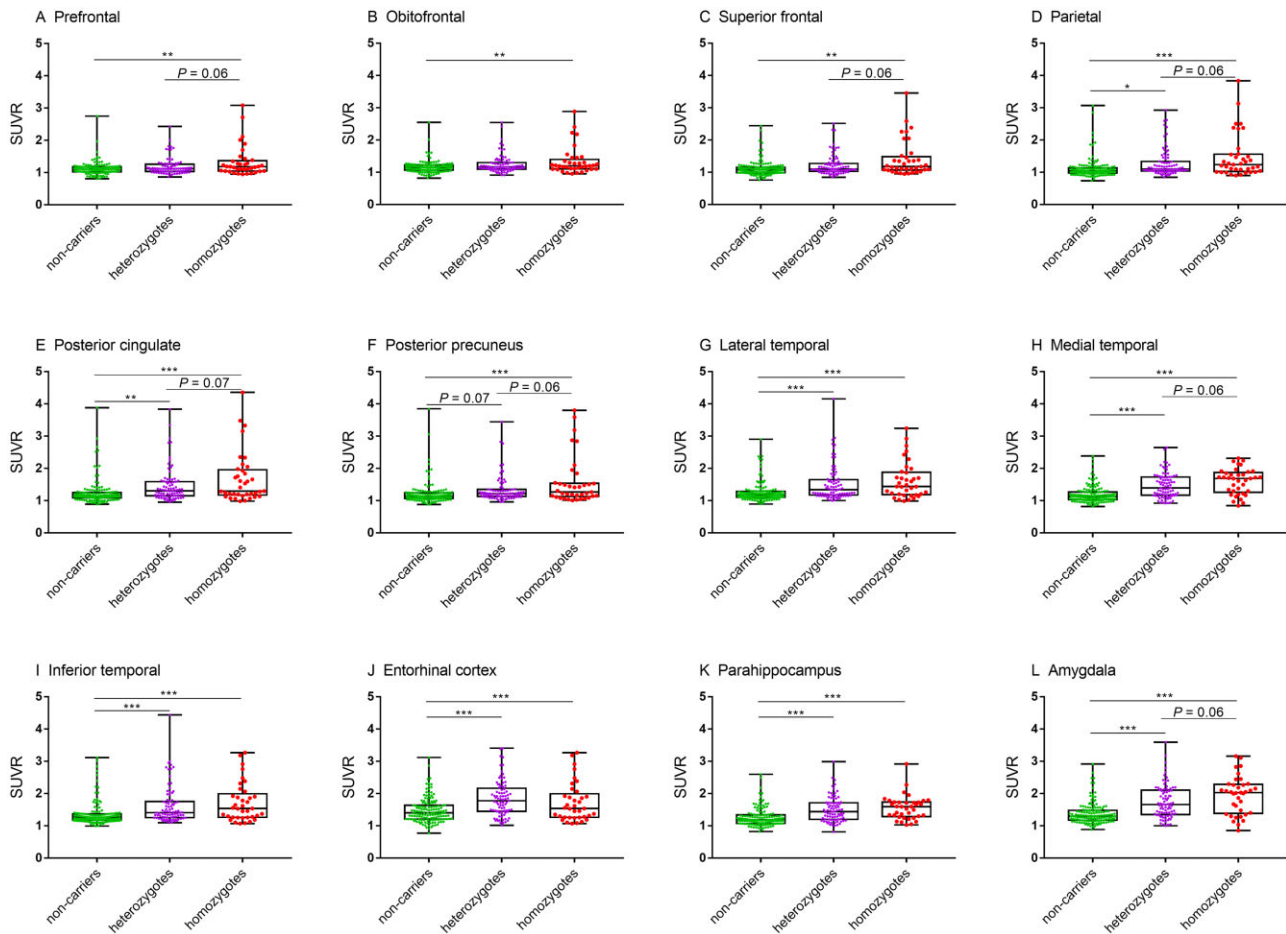


Figure 1 APOE $\epsilon 4$ dose effect on region of interest ^{18}F -flortaucipir SUVRs in the study cohort of cognitively impaired individuals. Mean (\pm SD) of SUVR for APOE $\epsilon 4$ non-carriers (green), heterozygotes (purple) and homozygotes (red) are depicted. P-value was defined using a generalized linear model, adjusting for age, years of education and sex. SUVR: Standardized uptake value ratio. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

shape in native space, the region of interest SUVRs were calculated on the SUVR images in standard MNI space.^{5,21,27,28}

Statistical analysis

All statistical analyses were performed using Statistical Analysis System (SAS v.9.4, SAS Institute, Inc.) and SPM12. A generalized linear model was used to evaluate group differences among APOE $\epsilon 4$ 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 $\epsilon 4 \times$ sex interaction effects on global cortical ^{18}F -florbetapir amyloid- β SUVR after controlling for age and years of education. This analysis revealed no significant APOE $\epsilon 4 \times$ 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 $\epsilon 4$ dosage \times sex interaction effects on regional brain tau deposition as described earlier on the region of interest level:

Model 1: ROI_SUVR (^{18}F -flortaucipir) \sim age + educational level + sex:
APOE $\epsilon 4$ dosage
Model 2: ROI_SUVR (^{18}F -flortaucipir) \sim age + educational level + glo-
bal cortex_SUVR (^{18}F -florbetapir) + sex: APOE $\epsilon 4$ dosage

The APOE $\epsilon 4$ dosage \times sex interaction effects on brain tau deposition was further evaluated by sex-stratified analysis. Specifically, group differences (i.e. APOE $\epsilon 4$ non-carriers,

heterozygotes and homozygotes) in ^{18}F -flortaucipir SUVR at the region of interest and voxel-wise 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 $\epsilon 4$ dosage groups. SAS was used for region of interest analyses and SPM12 ($P < 0.001$, cluster size > 100 voxels) was used for voxel-wise analysis as described previously.^{5,21,25} The Benjamini-Hochberg method was used to control the false discovery rate (FDR) using the 12 study regions of interest at both region of interest-based and voxel-wise levels. For region of interest-based analyses, an FDR corrected P-value < 0.05 was defined as significant. For voxel-wise based analyses, an FDR corrected value $P < 0.05$ and cluster size > 100 voxels were 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 individuals with cognitive impairment with ^{18}F -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

Table 2 APOE ε4 dosage × sex interaction effect on tau deposition in cognitively impaired participants

Region of interest	Not adjusted for global cortical amyloid level			Adjusted for global cortical amyloid level		
	Standardized β (95% CI)	APOE ε4 × Sex P-value	FDR P-value	Standardized β (95% CI)	APOE ε4 × Sex P-value	FDR P-value
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

P-value was defined using a generalized linear model to detect significant APOE ε4 dosage × 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 FDR; 95% CI represents the 95% confidence interval of the APOE ε4 dosage by sex coefficient.

amyloid-β positivity, CSF amyloid-β₄₂, 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 the female cohort, compared to non-carriers, APOE ε4 heterozygotes and homozygotes had decreased CSF amyloid-β₄₂ ($P < 0.01$), 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 the male cohort, compared to non-carriers, APOE ε4 homozygotes were younger ($P = 0.02$) and with a lower MMSE score ($P = 0.02$); APOE ε4 heterozygotes and homozygotes had decreased CSF amyloid-β₄₂ levels ($P < 0.01$); APOE ε4 heterozygotes had increased p-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 ($P = 0.64$) and 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 region of interest ¹⁸F-flortaucipir SUVRs in the prefrontal, superior frontal, parietal, posterior cingulate, posterior precuneus, medial temporal and amygdala (Fig. 1).

APOE ε4 dosage × sex interaction effect on regional ¹⁸F-flortaucipir SUVR

Regions of interest with significant APOE ε4 dosage × sex interaction on regional ¹⁸F-flortaucipir SUVR were identified with and without controlling for global cortical ¹⁸F-florbetapir SUVR (Table 2) using the 12 regions of interest described in the ‘Materials and methods’ section. We observed a significant APOE ε4 dosage × sex interaction effect on ¹⁸F-flortaucipir tau deposition in the lateral temporal, posterior cingulate, medial temporal, inferior temporal, entorhinal cortex, amygdala, parahippocampal gyrus regions without adjusting for global cortical ¹⁸F-florbetapir SUVR (FDR $P < 0.05$;

Table 2). The medial temporal, entorhinal cortex, amygdala and parahippocampal gyrus regions of interest retained significant APOE ε4 dosage × sex interaction effect on tau deposition after adjusting for global cortical ¹⁸F-florbetapir SUVR (FDR $P < 0.05$; Table 2).

Sex and APOE ε4 dosage-stratified analysis on regional ¹⁸F-flortaucipir SUVR

The ¹⁸F-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 region of interest-based SUVRs 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 regions of interest of medial temporal, entorhinal cortex and amygdala retained a significant difference between homozygotes and non-carriers after adjusting for global cortical ¹⁸F-florbetapir SUVR. Similarly, female heterozygotes had higher ¹⁸F-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 regions of interest of medial temporal, entorhinal cortex, amygdala and parahippocampal gyrus retained a significant difference between heterozygotes and non-carriers after adjusting for global cortical ¹⁸F-florbetapir SUVR (FDR $P < 0.05$). Strikingly, there were no significant differences in any of the 12 regions of interest SUVRs between female homozygotes and female heterozygotes with or without adjusting for global cortical ¹⁸F-florbetapir SUVR (FDR $P > 0.05$).

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

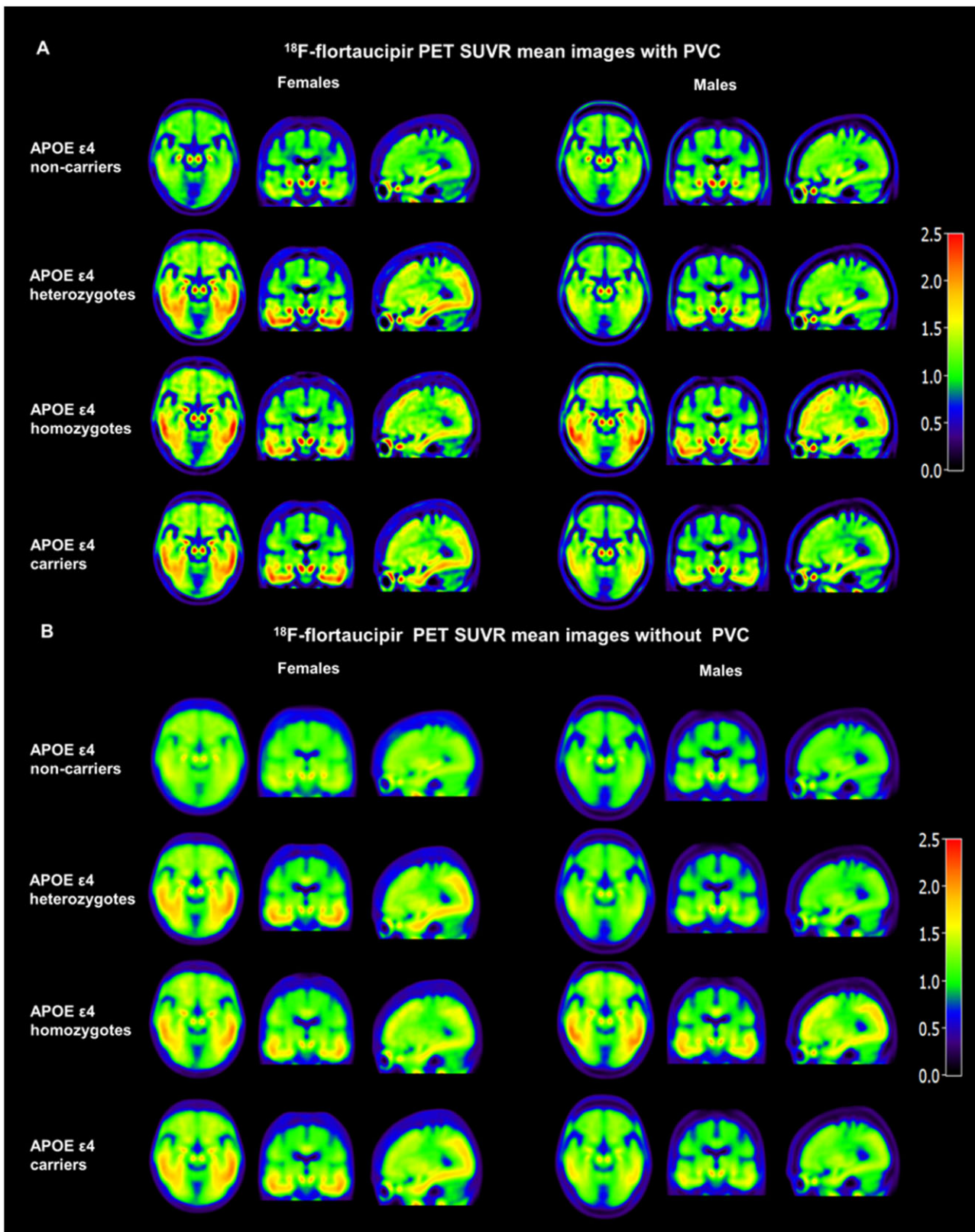


Figure 2 Mean images with and without PVC illustrate that sex modulates the APOE ϵ 4 dose effect on ^{18}F -flortaucipir SUVR in cognitively impaired participants. PVC images show (A) increased contrast and spatial resolution compared to images without PVC (B). Both PVC and non-PVC images visually demonstrate an interaction effect between sex and APOE ϵ 4 status.

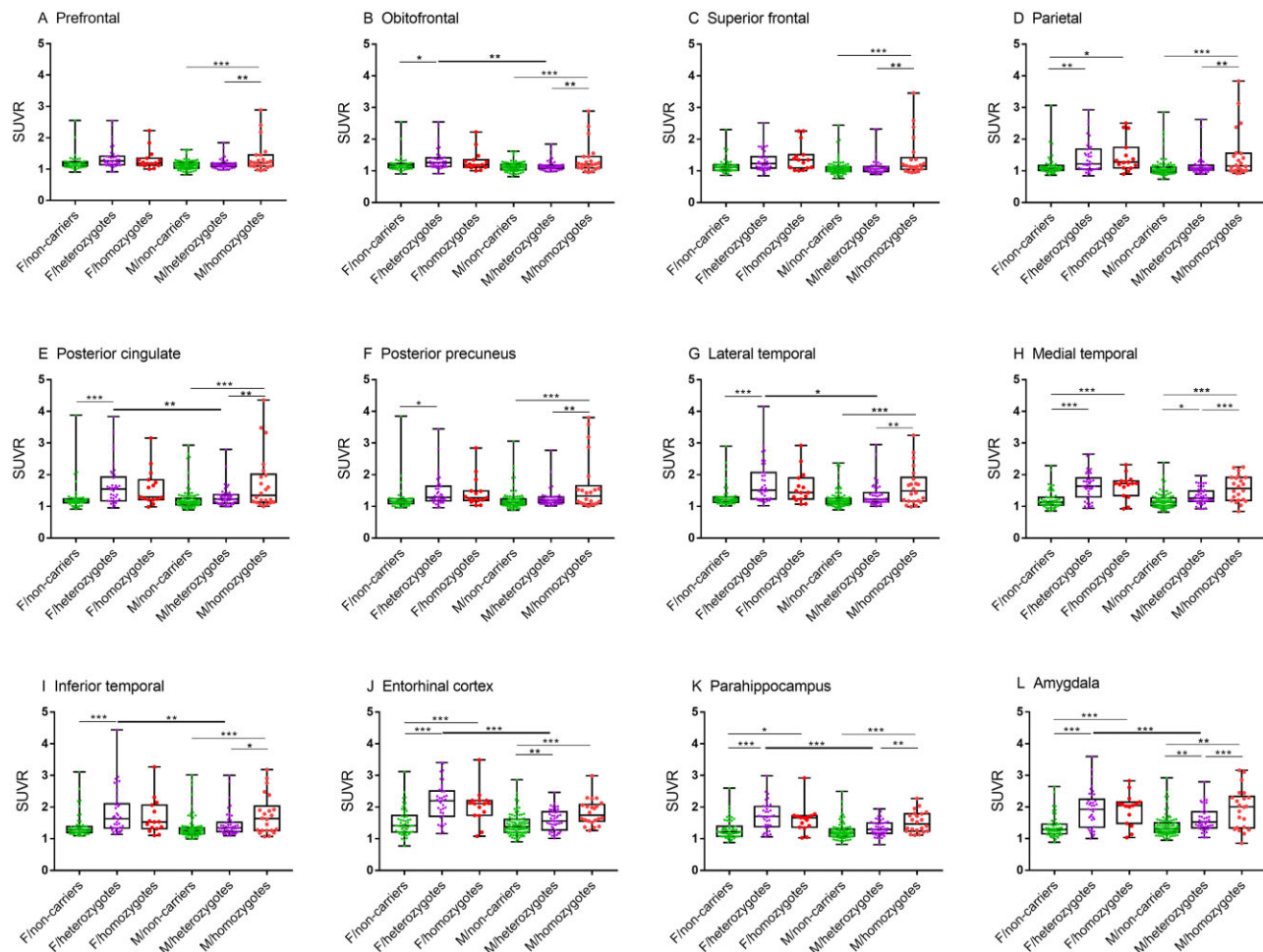


Figure 3 Sex modifies the APOE $\epsilon 4$ dosage effect on region of interest-based analysis of ^{18}F -flortaucipir PET in cognitively impaired individuals. Box plots depict median value and the interquartile ranges of regional SUVRs for APOE $\epsilon 4$ non-carriers (green), heterozygotes (purple) 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. Bold significance lines indicate comparison between APOE $\epsilon 4$ dosage groups. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

interest exhibited 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 region of interest ^{18}F -flortaucipir SUVRs between males and females for both homozygotes and non-carriers (*P* > 0.05).

Sex-stratified effect of APOE $\epsilon 4$ dosage on voxel-wise ^{18}F -flortaucipir SUVR analysis

Female APOE $\epsilon 4$ 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 and 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 $\epsilon 4$ 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 $\epsilon 4$ dosage-related tau deposition in cognitively impaired elderly individuals. Specifically, in females, increased tau deposition was observed in both APOE $\epsilon 4$ heterozygotes and homozygotes compared to non-carriers. But, in males,

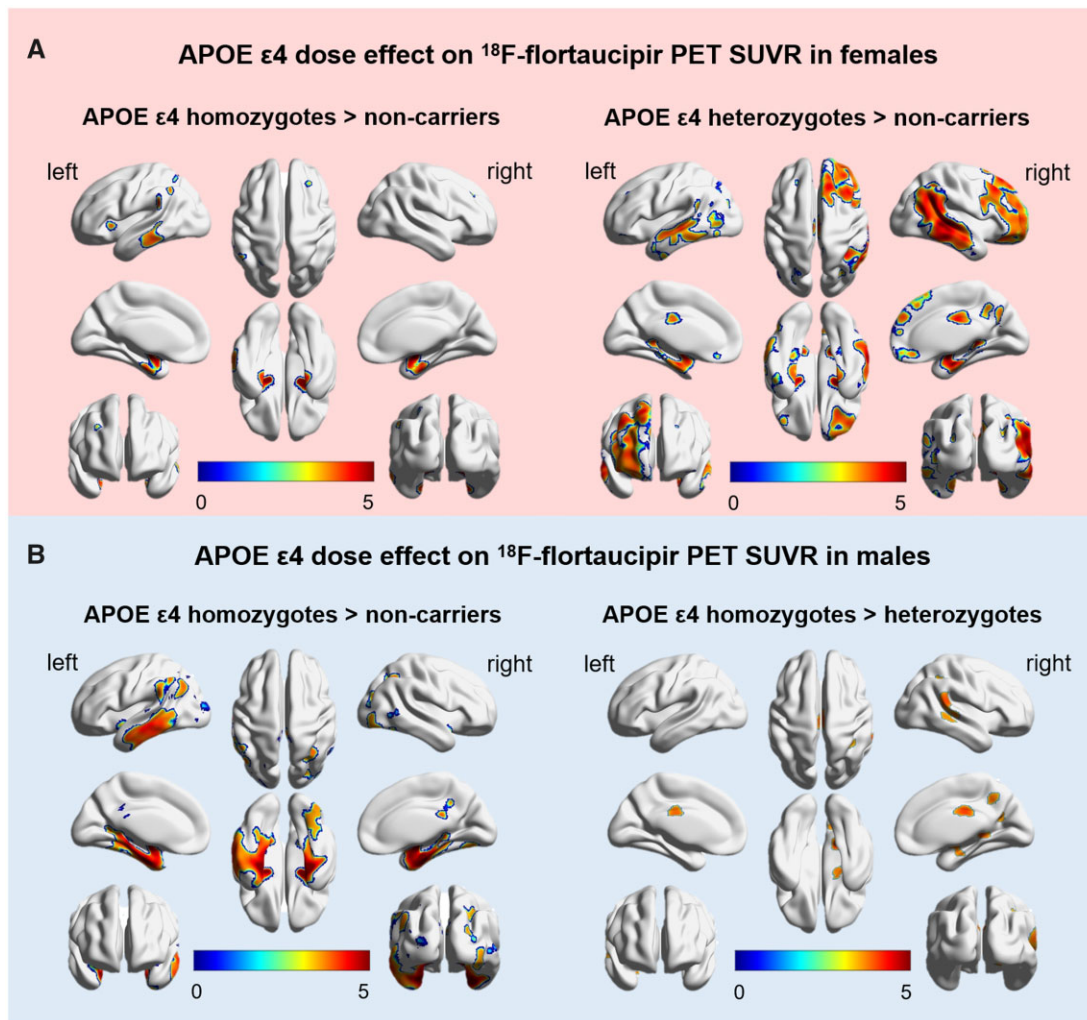


Figure 4 Sex modifies the APOE ε4 dose effect on voxel-wise analysis of ¹⁸F-flortaucipir PET in cognitively impaired participants. APOE ε4 dose effect on voxel-wise SUVR is depicted in (A) females and (B) males. Female heterozygotes (A, left) and homozygotes (A, right) display increased ¹⁸F-flortaucipir SUVR compared to non-carriers. No significant differences in SUVR were observed between female heterozygotes and homozygotes. Male homozygotes demonstrate increased ¹⁸F-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).

only the APOE ε4 homozygotes (and not the heterozygotes) had increased tau deposition 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 region of interest analysis of entorhinal cortex, amygdala and parahippocampal gyrus showed an amyloid-β-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 uncover a possible explanation underlying differential APOE ε4-associated Alzheimer's risk in males and females.

Our findings indicate 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 region of interest-based and voxel-wise

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 previous 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.^{1,2,31,32} Taken together, our data indicate that females may have higher susceptibility to the APOE ε4 allele than males. These findings are also consistent with work from Payami et al.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.³² Another study suggested that females have a higher risk of Alzheimer's disease than males not because of their greater longevity,³³ but probably due to the female-specific susceptibility among heterozygotes.^{2,32} Combined with these previous

Table 3 Clusters with significant association between ¹⁸F-flortaucipir SUVR and APOE ε4 dosage

Clusters	Female						Male					
	Homozygotes > non-carriers			Heterozygotes > non-carriers			Homozygotes > non-carriers			Homozygotes > heterozygotes		
	x	y	z	x	y	z	x	y	z	x	y	z
Temporal_Mid_L	-63	-28.5	-21	55	-66	0	-54	-26	-5	-	-	-
Temporal_Inf_L												
Temporal_Mid_R	-	-	-	55	-21	-21	58	-21	-7	60	-30	0
Temporal_Inf_R												
Temporal_Sup_R	-	-	-	64.5	-33	16.5	57	-6	2	-	-	-
ParaHippocampal_L	-18	-9	-18	-22	-12	-25	-18	-6	-21	-	-	-
Amygdala_L												
ParaHippocampal_R	18	-7.5	-18	18	-7.5	-18	18	-4	-22	-	-	-
Amygdala_R												
Cingulum_Mid_L	-	-	-	-6	-43	20	9	-27	36	-6	-22	36
Precuneus_L												
Cingulum_Mid_R	-	-	-	9	-43	26	9	-24	41	10.5	-51	45
Precuneus_R												
Parietal_Inf_L	-52.5	-52.5	42	-44	-48	40	-42	-48	43	9	-57	58.5
Parietal_Inf_R	34.5	-52.5	46.5	-	-	-	45	-53	53	33	-54	54
Parietal_Sup_L	-31.5	-63	60	-30	-67.5	57	-24	-67	51	-	-	-
Frontal_Mid_L	-	-	-	-	-	-	-4	59	17	-	-	-
Frontal_Sup_L												
Frontal_Mid_R	28.5	34.5	36	-	-	-	13.5	27	57	-	-	-
Frontal_Sup_R												

Data extracted from SPM12 analysis showing voxels with significantly increased ¹⁸F-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 Fig. 3. Atlas coordinates were obtained from Automated Anatomical Labelling (AAL).⁴⁰

findings and results for the study, we provide a potential genetic-informed explanation underlying increased susceptibility to Alzheimer's disease in females.³⁴

Alzheimer's disease pathology is characterized by the accumulation of amyloid-β plaques and neurofibrillary tangles in the brain. Our study found an amyloid-β-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 × sex interaction effect on tau deposition were partially mediated by amyloid-β. In line with this, a biochemical study found that accumulations of tau and amyloid-β occur independently in the entorhinal cortex.³⁵ Two recent cross-sectional tau PET studies indicated that APOE ε4 is associated with tau deposition in the medial temporal cortex independent of amyloid-β status.^{30,36} The neocortex amyloid-β-dependent tau deposition maybe explained by the dual-cascade hypothesis.³⁷ However, our results are in contrast with a recent longitudinal tau PET study, which showed that the accumulation rate of tau in temporal meta- and neocortical regions of interest is increased in females amyloid-β-positive individuals.³⁸ It should be noted that the analyses with controlling for global cortical ¹⁸F-florbetapir SUVR were performed in a subset of the cohort of 188 individuals. Therefore, the reduced regions of interest of APOE ε4 dosage × sex interaction effect after controlling for ¹⁸F-florbetapir SUVR may be partly explained by differences in sample size. We realized that the time interval between the ¹⁸F-florbetapir and ¹⁸F-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 time interval of the two scans.

Past reports have demonstrated that the effect of APOE ε4 on CSF t-tau and p-tau levels is stronger in females than males.¹⁴ Sundermann *et al.*¹⁷ showed that APOE ε4 is associated with greater

amyloid-β burden across males and females in individuals with mild cognitive impairment, 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.^{5,16} This lack of concordance of APOE ε4 dose effects between brain ¹⁸F-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.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 previous study, APOE ε4 homozygotes may have an increased risk of Alzheimer's disease before their seventies, but resilience to Alzheimer's disease beyond their seventies.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 size. The statistical power for ¹⁸F-flortaucipir SUVR to distinguish between APOE ε4 heterozygotes and homozygotes ranged from 0.97 to 0.999 in all 12 regions of interest 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 going ADNI projects and other larger cohort studies.

In conclusion, this quantitative ^{18}F -flortaucipir PET study in individuals with cognitive impairment showed that an APOE $\epsilon 4$ 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 $\epsilon 4$ dose effect in biomarker development and mechanistic studies in Alzheimer's disease using quantitative ^{18}F -flortaucipir PET.

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