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Sex modifies APOE ε4 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* 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 ^{18}F-flortaucipir PET.

Preprocessed ^{18}F-flortaucipir tau PET images, T_1-weighted structural MRI, demographic information, global cortical amyloid-β burden measured by ^{18}F-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. 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* ε4 dosage × sex interaction effect on ^{18}F-flortaucipir standardized uptake value ratios was assessed using generalized linear models and sex-stratified analysis.

We observed a significant *APOE* ε4 dosage × 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* ε4 dosage × 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* ε4 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* ε4-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*) 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.

^{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* 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 and Alzheimer's disease.[5–10] Similarly, *APOE* ε4 carriers show accelerated brain amyloid-β 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 ^{18}F-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.[13]

Previous studies have suggested that *APOE* ε4 confers a greater risk for Alzheimer's disease, tau pathology, glucose hypometabolism and amyloid-β 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.[2] In regard 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.[14–16] A PET study found that while *APOE* ε4 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* ε4 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* ε4 carriers compared to other groups.[18] Further, we have previously elucidated a sex × *APOE* ε4 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* ε4 genotype was analysed 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 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 × APOE ε4 carrier status interaction effect on tau pathology, a sex × *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 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* ε2/ε4 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 $A\beta_{42}$ (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 ^{18}F-florbetaben cortical SUVR based on the whole cerebellum reference = 1.08 and ^{18}F-florbetapir = 1.11 (adni.loni.usc.edu/methods).

[a]$P < 0.05$ compared to non-carriers.

[b]$P < 0.01$ 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-β_{42} 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 T_1-weighted structural MRI and preprocessed ^{18}F-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

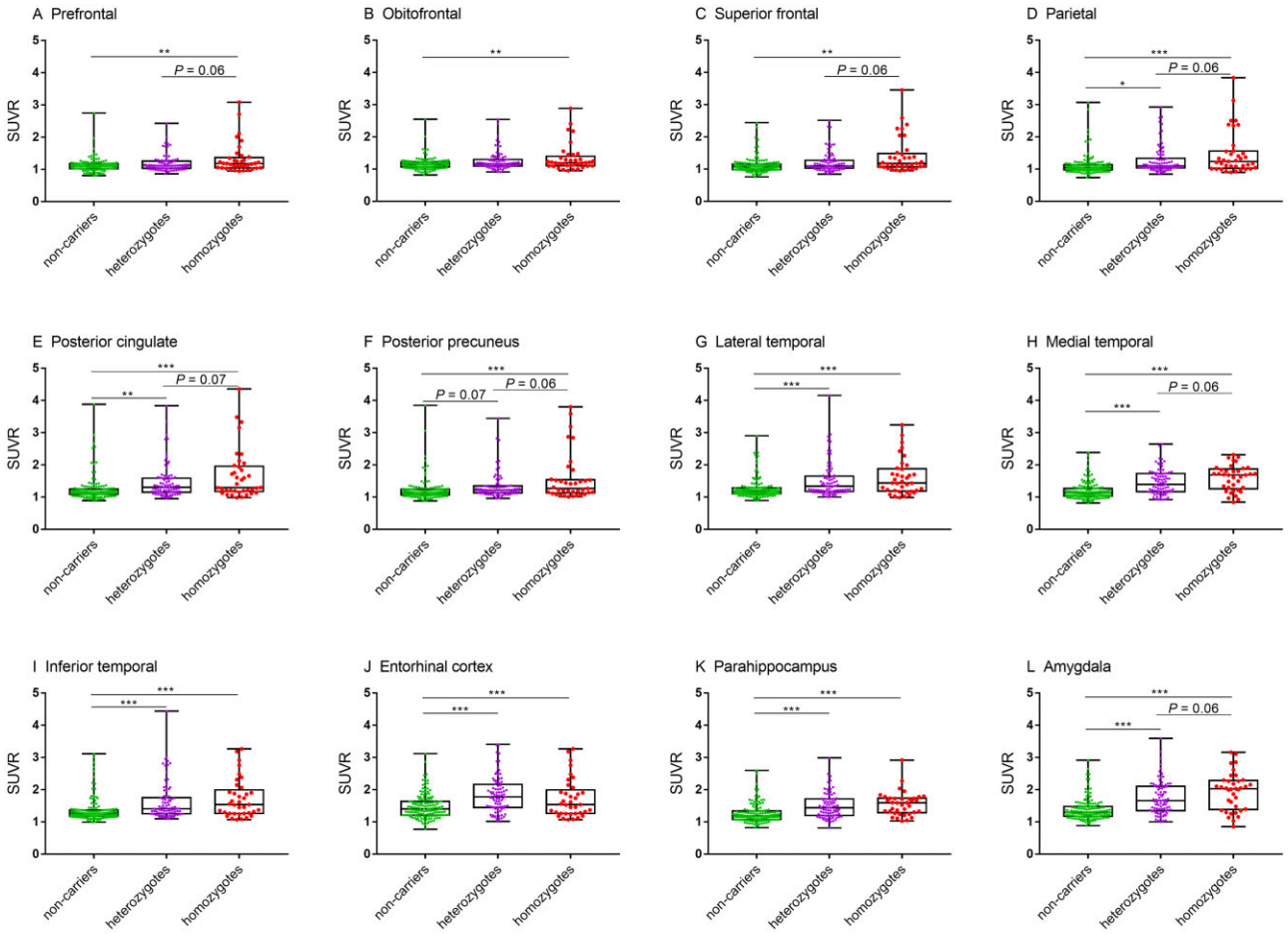

Figure 1 *APOE* ε4 dose effect on region of interest ^{18}F-flortaucipir SUVRs in the study cohort of cognitively impaired individuals. Mean (± SD) of SUVR for *APOE* ε4 non-carriers (green), heterozygotes (purple) and homozygotes (red) are depicted. *P*-value was defined using a generalized linear model, 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* ε4 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* ε4 × 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 ε4 × 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* ε4 dosage × sex interaction effects on regional brain tau deposition as described earlier on the region of interest level:

Model 1: ROI_SUVR (^{18}F-flortaucipir) ~ age + educational level + sex: *APOE* ε4 dosage
Model 2: ROI_SUVR (^{18}F-flortaucipir) ~ age + educational level + global cortex_SUVR (^{18}F-florbetapir) + sex: *APOE* ε4 dosage

The *APOE* ε4 dosage × sex interaction effects on brain tau deposition was further evaluated by sex-stratified analysis. Specifically, group differences (i.e. *APOE* ε4 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* ε4 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 ^{18}F-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-β_{42}, 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-β_{42} ($P < 0.01$), 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-β_{42} 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 ^{18}F-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 ^{18}F-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 ^{18}F-flortaucipir SUVR

Regions of interest with significant *APOE* ε4 dosage × 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 regions of interest described in the 'Materials and methods' section. We observed a significant *APOE* ε4 dosage × 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 $P < 0.05$; 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 ^{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 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 ^{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 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 ^{18}F-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 ^{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 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 ^{18}F-florbetapir SUVR. In addition, male heterozygotes had higher ^{18}F-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

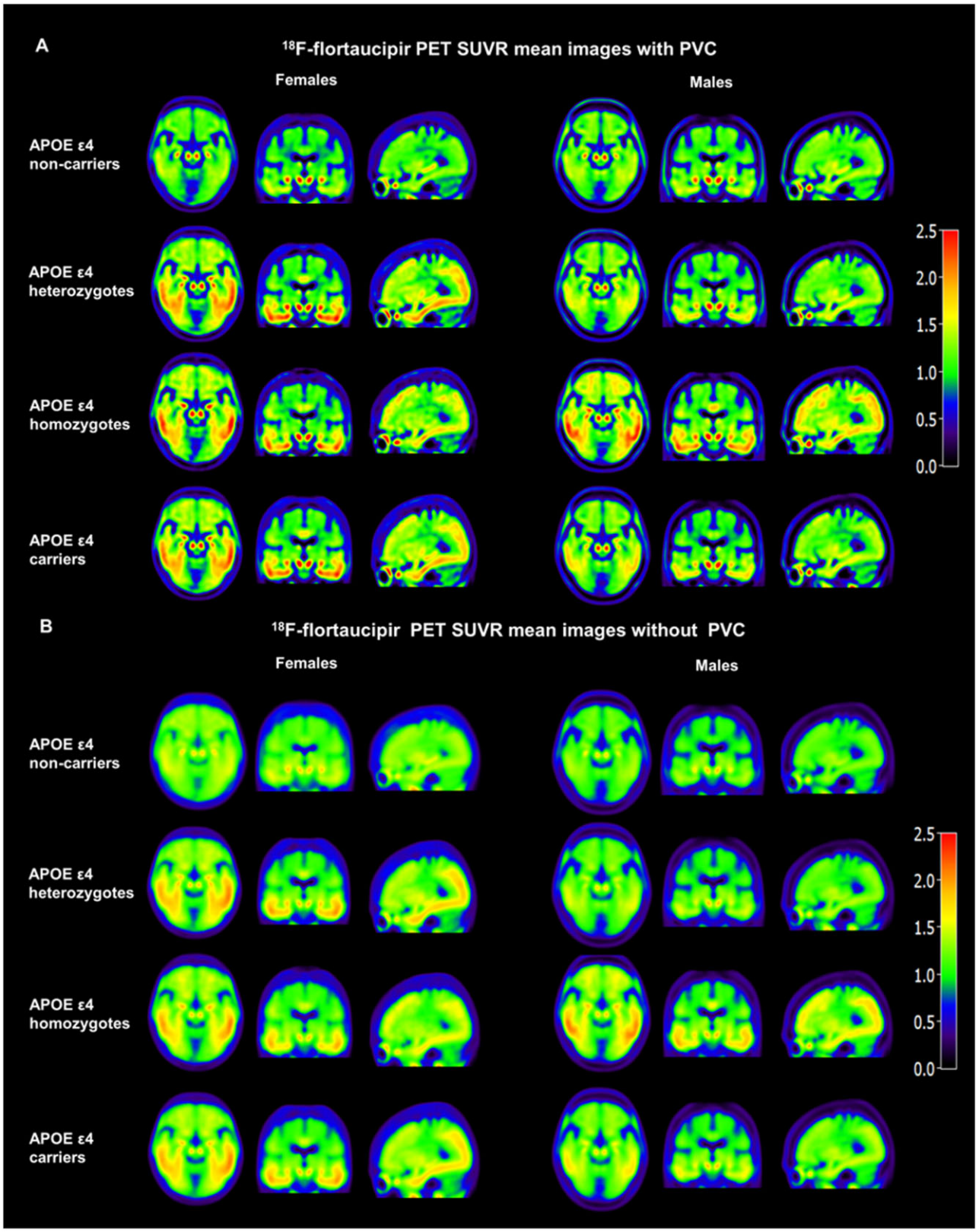

Figure 2 Mean images with and without PVC illustrate that sex modulates the *APOE* ε4 dose effect on ^{18}F-flortaucipir SUVR in cognitively impaired participants. PVC images show (**A**) increased contrast and spatial resolution compared to images without PVC (**B**). Both PVC and non-PVC images visually demonstrate an interaction effect between sex and *APOE* ε4 status.

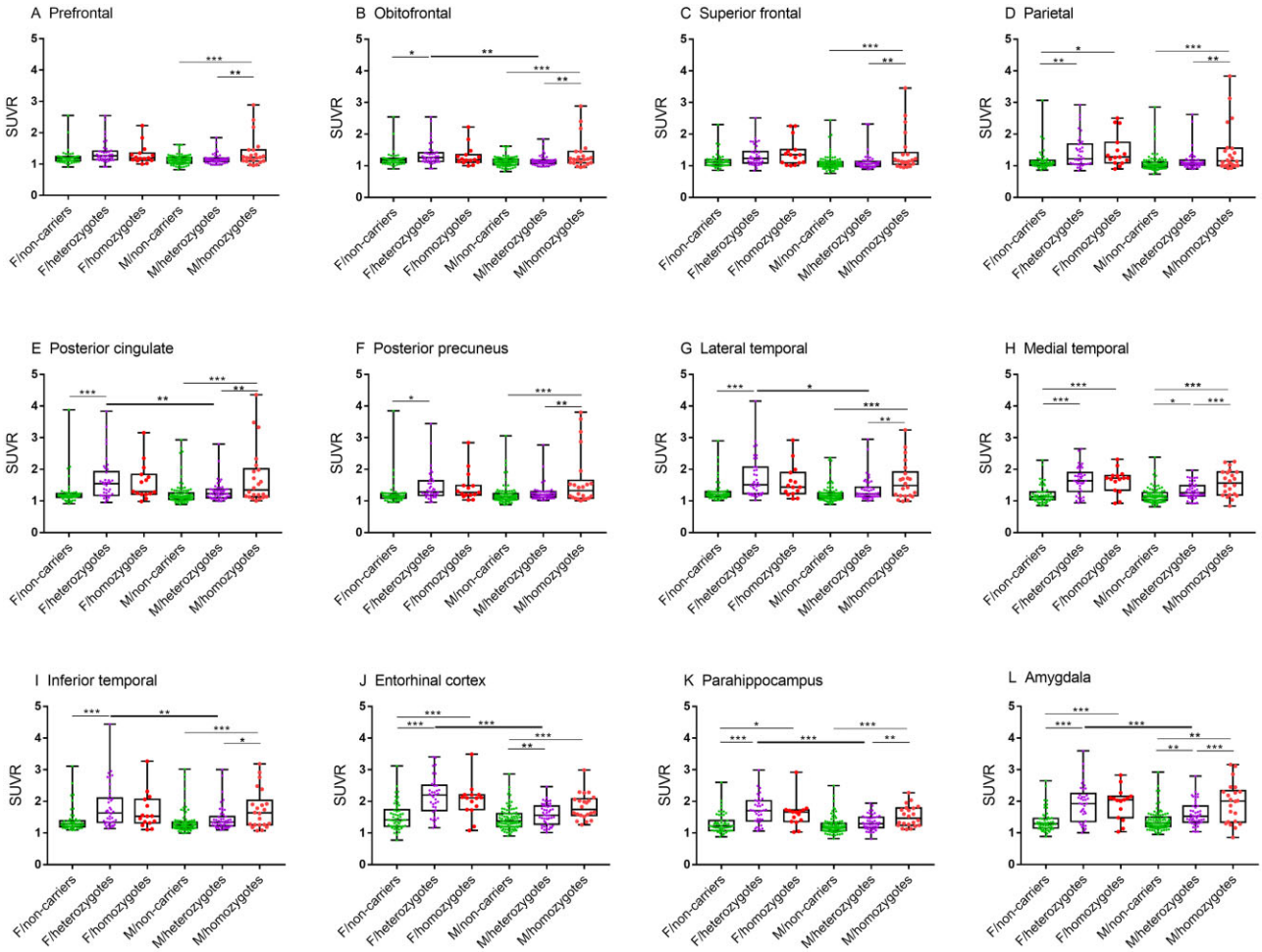

Figure 3 Sex modifies the *APOE* ε4 dosage effect on region of interest-based analysis of ^{18}F-flortaucipir PET in cognitively impaired individuals. Box plots depict median value and the interquartile ranges of regional SUVRs for *APOE* ε4 non-carriers (green), heterozygotes (purple) and homozygotes (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. Bold significance lines indicate comparison between *APOE* ε4 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* ε4 dosage on voxel-wise ^{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 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* 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,

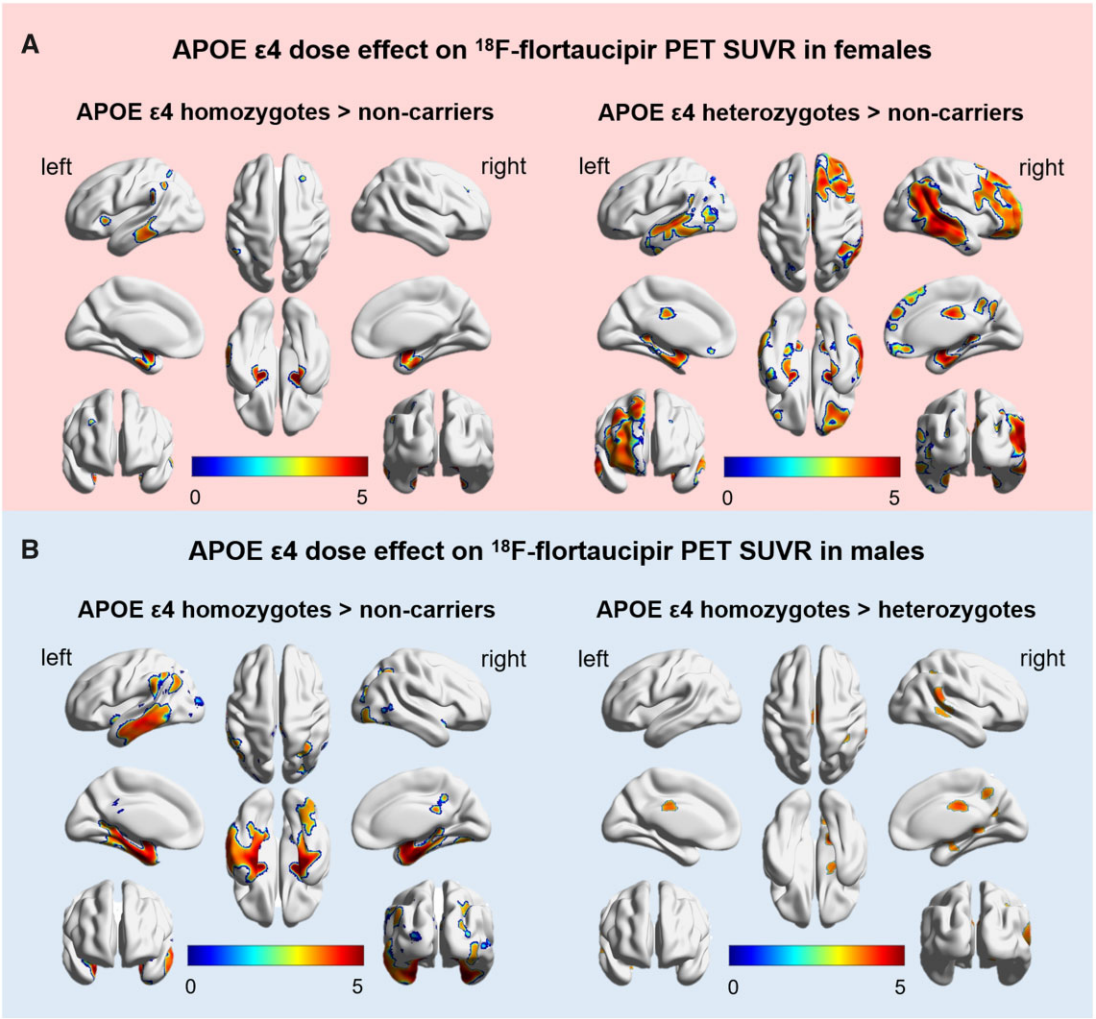

Figure 4 Sex modifies the *APOE* ε4 dose effect on voxel-wise analysis of ^{18}F-flortaucipir PET in cognitively impaired participants. *APOE* ε4 dose effect on voxel-wise SUVR is depicted in (**A**) females and (**B**) males. Female heterozygotes (**A**, *left*) and homozygotes (**A**, *right*) display increased ^{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).

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 ^{18}F-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 ^{18}F-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 ^{18}F-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 Temporal_Inf_L	–63	–28.5	–21	55	–66	0	–54	–26	–5	–	–	–
Temporal_Mid_R Temporal_Inf_R	–	–	–	55	–21	–21	58	–21	–7	60	–30	0
Temporal_Sup_R	–	–	–	64.5	–33	16.5	57	–6	2	–	–	–
ParaHippocampal_L Amygdala_L	–18	–9	–18	–22	–12	–25	–18	–6	–21	–	–	–
ParaHippocampal_R Amygdala_R	18	–7.5	–18	18	–7.5	–18	18	–4	–22	–	–	–
Cingulum_Mid_L Precuneus_L	–	–	–	-6	–43	20	9	–27	36	–6 –	–22 –	36 –
Cingulum_Mid_R Precuneus_R	–	–	–	9	–43	26	9	–24	41	10.5	–51	45
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 Frontal_Sup_L	–	–	–	–	–	–	–4	59	17	–	–	–
Frontal_Mid_R Frontal_Sup_R	28.5	34.5	36	–	–	–	13.5	27	57	–	–	–

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 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 ^{18}F-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 ^{18}F-florbetapir SUVR may be partly explained by differences in sample size. We realized that the time interval between the ^{18}F-florbetapir and ^{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 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 ^{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.[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 ^{18}F-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* 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 ^{18}F-flortaucipir PET.

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