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RESEARCH ARTICLE

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Association between long-term donepezil treatment and brain regional amyloid and tau burden among individuals with mild cognitive impairment assessed using ^{18}F-AV-45 and ^{18}F-AV-1451 PET

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Abstract

This study aims to investigate the association between long-term donepezil treatment and brain neuropathological burden and cognitive function in mild cognitive impairment (MCI) patients. Preprocessed ^{18}F-AV-45 amyloid and ^{18}F-AV-1451 tau positron emission tomography (PET) images, magnetic resonance imaging images (MRIs), demographic information, and donepezil use status were downloaded from 255 MCI participants enrolled in the Alzheimer's Disease Neuroimaging Initiative database. Partial volume correction was applied to all PET images. Structural MRIs were used for PET spatial normalization. Regions of interest (ROIs) were defined in standard space, and standardized uptake value ratio (SUVR) images relative to the cerebellum were computed. Multiple linear regression with the least absolute shrinkage selector operator was performed to analyze the effect of long-term donepezil treatment on (a) the SUVR of each ^{18}F-AV-45 or ^{18}F-AV-1451 brain PET ROI after adjusting for age, sex, education, ApoE ε4 status, and AD-associated disease risk factors; and (b) cognitive performance after adjusting for age, sex education, ApoE ε4 status, AD-associated disease risk factors, and regional amyloid or tau burden. In adjusted models, long-term donepezil treatment was associated with greater amyloid load in the orbital frontal, superior frontal, parietal, posterior precuneus, posterior cingulate, lateral temporal, inferior temporal and fusiform regions, and tau burden in the posterior cingulate, entorhinal and parahippocampal gyrus. Long-term donepezil treatment was also associated with worse performance on the 13-item Alzheimer's Disease Assessment Scale-Cognitive subscale after adjusting for AD-related risk factors and regional brain amyloid or tau load. These results indicate that long-term

Abbreviations: Aβ, amyloid β; AD, Alzheimer's disease; ADAS-Cog 13, Alzheimer's Disease Assessment Scale-Cognitive subscale 13; ADNI, Alzheimer's Disease Neuroimaging Initiative; ApoE ε4, apolipoprotein E type 4 allele; ChEI, cholinesterase inhibitor; FWHM, full width at half maximum; LASSO, least absolute shrinkage selector operator; MCI, mild cognitive impairment; MNI, Montreal Neurological Institute; MRI, magnetic resonance imaging; PET, positron emission tomography; PVC, partial volume correction; ROIs, regions of interest; SAS, Statistical Analysis System; SPM, statistical parametric mapping; SUVR, standardized uptake value ratio.

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Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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donepezil treatment is associated with increased regional amyloid and tau burden and worse cognitive performance among individuals with MCI. Our study highlights the importance of using noninvasive and quantitative ^{18}F-AV-45 and ^{18}F-AV-1451 PET to elucidate the consequences of drug administration in AD studies.

KEYWORDS
Alzheimer's disease, amyloid β, donepezil, PET, tau

1 | INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disorder and is driven by multiple etiologies including unmodifiable factors (e.g., age, sex, and ApoE ε4 status) and modifiable factors, including diabetes mellitus, hypertension, hyperlipidemia, and low levels of education (Kivipelto et al., 2018; Sharma et al., 2019). Neuropathological hallmarks of AD include intracellular neurofibrillary tangles comprised of hyperphosphorylated tau protein and extracellular amyloid fibrils and plaques.

Donepezil, a selective cholinesterase inhibitor (ChEI), is the most widely used medicine to improve cognitive symptoms in individuals with mild to moderate dementia and mild cognitive impairment (MCI) (Han et al., 2019; Roberts et al., 2010). Despite its widespread use, previous studies examining the effect of donepezil treatment on neuropathological changes and cognitive function in MCI individuals have been inconsistent. For example, some studies have found that donepezil exerts a protective effect by attenuating neural amyloid β (Aβ) toxicity and influencing amyloid precursor protein (APP) processing (Jacobson & Sabbagh, 2008), reducing glutamate neurotoxicity and inhibiting excitotoxic injury (Takada-Takatori et al., 2006), and suppressing oxidative stress (Saxena et al., 2008). In contrast, other studies have found that donepezil exposure may increase brain tau and Aβ burden (Chalmers et al., 2009; Ishibashi et al., 2017). Some clinical trials aimed at assessing the effect of donepezil on cognitive function have demonstrated improvements in the Alzheimer's Disease Assessment Scale-Cognitive (ADAS-cog) total score, patient global cognitive assessment, tests of attention and psychomotor speed, and delayed progression to AD in MCI participants (Doody et al., 2009; Petersen et al., 2005; Salloway et al., 2004). However, other trials have failed to find an effect of donepezil on cognitive function (Han et al., 2019; Schneider et al., 2011). A greater understanding of the effect of donepezil on pathological and cognitive changes in individuals with MCI is sorely needed.

The goal of this study was to elucidate the effects of long-term donepezil treatment on neuropathological changes and global cognitive function in MCI individuals from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. The results outlined in this study will provide further support for the use of ^{18}F-AV-45 and ^{18}F-AV-1451 positron emission tomography (PET) as quantitative readouts of efficacy in future AD clinical trials. Further, the insights learned from this work will advance our understanding of the neuropathological effects of donepezil and form a paradigm for the use of PET imaging as a noninvasive tool to study the *in vivo* consequences of drug administration.

Significance

Donepezil, a selective cholinesterase inhibitor, is the most widely used medicine to improve cognitive symptoms in individuals with mild to moderate dementia and mild cognitive impairment (MCI). To the best of our knowledge, this is the first study investigating the effects of long-term donepezil treatment on brain region-specific amyloid and tau deposition in patients with MCI. Our findings suggest that long-term donepezil treatment is associated with greater regional amyloid and tau burden and worse cognitive performance among individuals with MCI.

2 | METHODS

2.1 | Participants

Data used in this study were obtained from the ADNI database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public–private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI is to test whether serial magnetic resonance imaging (MRI), PET, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. Clinical metadata including age, sex, years of education, ApoE ε4 status, initial health assessment, medication history, global cognitive assessment, and imaging data including ^{18}F-AV-45 PET, ^{18}F-AV-1451 PET, and T1-weighted structural MRI scans were downloaded. MCI participants were identified using inclusion criteria as detailed by ADNI consortium investigators. Specifically, all MCI participants included in this study had a subjective memory concern as reported by a clinician or study partner, objective memory loss measured using education-adjusted scores on the Logical Memory II (Delayed Recall) subscale of the Wechsler Memory Scale, a clinical dementia rating (CDR) of 0.5, and general cognition sufficiently preserved such that a diagnosis of dementia could not be made. MCI

participants were classified as donepezil users or nonusers of ChEI (including donepezil, rivastigmine, and galantamine) before their most recent ^{18}F-AV-45 or ^{18}F-AV-1451 PET scan. Donepezil users were defined as MCI participants who met the following criteria: (a) had received at last one ^{18}F-AV-45 or ^{18}F-AV-1451 PET scan, (b) had a matched T1 MRI, (c) completed a detailed clinical phenotyping questionnaire, and (d) reported taking donepezil before receiving an ^{18}F-AV-45 or ^{18}F-AV-1451 PET scan. ChEI nonusers were defined as individuals who had (a) received an ^{18}F-AV-45 or ^{18}F-AV-1451 PET scan, (b) matched T1 MRI, (c) completed a detailed clinical phenotyping questionnaire, and (d) no ChEI use history prior to receiving an ^{18}F-AV-45 or ^{18}F-AV-1451 PET scan. Other ADNI exclusion/inclusion procedures can be found at https://adni.loni.usc.edu/wp-content/uploads/2008/07/adni2-procedures-manual.pdf. Due to the majority of donepezil users having an exposure time greater than 6 months before receiving an ^{18}F-AV-45 or ^{18}F-AV-1451 PET scan, we excluded donepezil users with exposure <6 months to select for only long-term donepezil users. A total of 127 MCI participants with an ^{18}F-AV-45 PET scan (donepezil users 31 and ChEI nonusers 96) and 128 MCI participants with an ^{18}F-AV-1451 PET scan (donepezil users 23 and ChEI nonusers 105) were included in the study.

Informed consent was obtained from all study participants at the time of enrollment for imaging data, genetic sample collection, and clinical questionnaires by ADNI study personnel.

2.2 | ApoE genotyping

Peripheral blood (10 ml) was collected from study participants for ApoE ε4 genotyping. Restriction enzyme isoform genotyping was used on DNA extracts to test for the presence of the ApoE ε4 genotype, as described previously (Hixson & Vernier, 1990). ApoE ε4 carriers were defined as individuals with at least one ε4 allele (either ε4/ε4, ε4/ ε3, or ε4/ ε2). Noncarriers were defined as individuals with no ε4 allele.

2.3 | Medication use history

The donepezil use history of MCI participants was extracted from the ADNI file "RECCMEDS.csv."

2.4 | Cognitive evaluation and attainment of risk factors

The 13-item Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog 13, in which higher scores indicate worse performance) was used to assess global cognition. History of common AD-related modifiable diseases, including hypercholesterolemia, hypertension, and diabetes were acquired from the ADNI file "INITHEALTH.csv."

2.5 | MRI and PET acquisition and processing

T1-weighted MRI and preprocessed ^{18}F-AV-45 and ^{18}F-AV-1451 PET images were downloaded from the ADNI database (http://adni.loni.usc.edu/). The PET images had been previously aligned, averaged, reoriented, and then interpolated into a standard image and voxel size (image volume 160 × 160 × 96, 1.5 × 1.5 × 1.5 mm in *x*, *y*, *z*), and smoothed to a uniform resolution of 8 mm in full width at half maximum (FWHM) by the ADNI consortium. We further processed the downloaded PET images using Statistical Parametric Mapping (SPM12, Wellcome Department of Imaging Neuroscience, London, United Kingdom) and MATLAB R2019b (The MathWorks Inc.). Briefly, all PET images were coregistered to structural MRI images. The MRI images were normalized to standard Montreal Neurologic Institute (MNI) space using SPM12 and VBM8 toolbox with an MRI template (image volume: 121 × 145 × 121, voxel size: 1.5 × 1.5 × 15 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. Regions of interest (ROIs) including

TABLE 1 Clinical and demographic characteristics of study participants

	Participants with ^{18}F-AV-45			Participants ^{18}F-AV-1451		
	Users (*n* = 31)	Nonusers (*n* = 96)	Statistics	Users (*n* = 23)	Nonusers (*n* = 105)	Statistics
Age, mean (*SD*), years	74.73 (6.94)	75.26 (7.89)	$t(125) = 0.336$, $p = 0.737$	76.01 (9.03)	75.88 (8.17)	$t(126) = -0.072$, $p = 0.943$
Sex, F/M	12/19	41/55	$\chi^2(1) = 0.034$, $p = 0.855$	7/16	40/65	$\chi^2(1) = 0.204$, $p = 0.652$
Education, mean (*SD*), years	15.55 (6.94)	16.06 (2.74)	$t(125) = 0.881$, $p = 0.380$	16.09 (3.40)	16.14 (2.69)	$t(126) = 0.086$, $p = 0.932$
ApoE ε4, P/N	14/17	37/59	$\chi^2(1) = 0.196$, $p = 0.658$	11/12	36/69	$\chi^2(1) = 0.963$, $p = 0.326$
Hypertension, Y/N	11/20	36/60	$\chi^2(1) = 0.000$, $p = 1.000$	11/12	39/66	$\chi^2(1) = 0.511$, $p = 0.474$
Hyperlipidemia, Y/N	15/16	37/59	$\chi^2(1) = 0.576$, $p = 0.448$	10/13	51/54	$\chi^2(1) = 0.212$, $p = 0.645$
Diabetes, Y/N	3/28	11/85	$p = 1.000$	3/20	12/93	$p = 0.733$

Note: Fisher's exact test was used for the comparison of diabetes rate between donepezil users and ChEI nonusers.
Abbreviations: ChEI, cholinesterase inhibitor; F/M, female/man; P/N, positive/negative; Y/N, yes/no.

TABLE 2 Final multiple linear regression models showing the association between long-term donepezil use and regional amyloid β deposition

	Regression coefficient β_i (95%CI), p-value of β_i			
ROI	β_0	Age	Sex	Education
Orbital frontal	0.63	0.01 (0.00–0.01), **$p = 0.049$**	–	–
Superior frontal	1.17	–	–	–
Lateral temporal	0.58	0.01 (0.00–0.01), **$p = 0.015$**	−0.05 (−0.13,0.04), $p = 0.267$	–
Parietal	0.65	0.01 (0.00–0.01), $p = 0.058$	–	–
Posterior precuneus	0.48	0.01 (0.00–0.02), **$p = 0.009$**	–	–
Posterior cingulate	0.30	0.01 (0.00–0.02), **$p = 0.004$**	–	–
Inferior temporal	0.85	0.01 (0.00–0.01), **$p = 0.034$**	−0.05 (−0.13,0.03), $p = 0.262$	–
Fusiform	0.88	0.00 (0.00–0.01), $p = 0.081$	–	−0.01 (−0.02,0.01), $p = 0.216$

Notes: All statistically significant p values are bolded.

Multiple linear regression model: SUVR(ROI) = $\beta_0 + \beta_1$ age + β_2 sex + β_3 education + β_4 ApoE ε4 + β_5 hyperlipidemia + β_6 donepezil. The SUVR (ROI) was measured by ^{18}F-AV-45 amyloid PET. β_i = - indicates a covariate that was not included in the final model for the ROI.

Abbreviations: adjusted R^2, adjusted R square of the final multiple linear regression model; p-value of regression, p-value of the final multiple linear regression model; 95% CI, 95% confidence interval.

cerebellum gray matter for reference tissue were manually drawn on the MRI template using PMOD (version 4.002 PMOD Technologies Ltd., Zürich, Switzerland) in standard MNI space.

In addition to cerebellum reference tissue ROI, 13 amyloid or tau pathologic-related brain ROIs were also defined in MNI space including: the entorhinal cortex, amygdala, fusiform, parahippocampal gyrus, occipital, inferior temporal, middle temporal, lateral temporal, parietal, posterior precuneus, orbital frontal, superior frontal, and posterior cingulate cortex (Gottesman et al., 2016; Liu et al., 2019; Paranjpe et al., 2019; Resnick et al., 2010; Zhou et al., 2007). All of these 13 regions were either previously determined by our group to significantly differ in ^{18}F-AV-1451 PET standardized uptake value ratio (SUVR) between cognitively normal, MCI, and AD patients (Zhao et al., 2019) or were required to define global cortical amyloid, as previously described (Gottesman et al., 2016).

A partial volume correction (PVC) was applied to the processed ^{18}F-AV-45 and ^{18}F-AV-1451 PET images to correct or minimize potential underestimation in PET measurement due to low image resolution, especially for small regions as the amygdala and striatum. In brief, an iterative reblurred Van Cittert iteration method was used for PVC on the mean images, where a 3D Gaussian kernel of 8 mm FWHM was used for spatial smoothing function h, step length $\alpha = 1.5$, and the iteration was stopped if relative percent change of PVC images <1% (Tohka & Reilhac, 2008). SUVR images were calculated relative to the cerebellum. ROI SUVRs were obtained by calculating mean SUVR within ROIs on the SUVR images in the MNI space.

2.6 | Statistical analysis

Differences in age, sex, education, ApoE ε4 status, and the prevalence of common AD-related modifiable disease risk factors (Table 1) between donepezil users and ChEI nonusers were assessed using Pearson's chi-squared test (or Fisher's exact test) for categorical variables and Student's t test for continuous variables. $p < 0.05$ was deemed significant.

Multiple linear regression models with least absolute shrinkage selector operator (LASSO) were carried out to test independently the effect of long-term donepezil treatment (>6 months) on the SUVR of each of 13 preselected ^{18}F-AV-45 and ^{18}F-AV-1451 PET brain ROIs after adjusting for age, sex, education, ApoE ε4 status, and common modifiable disease risk factors including history of hypertension, hyperlipidemia, and diabetes. Multiple linear regression with LASSO was also performed to test independently the effect of long-term donepezil treatment on cognitive performance after adjusting for age, sex, education, ApoE ε4 status, modifiable disease risk factors including history of hypertension, hyperlipidemia, and diabetes, and ^{18}F-AV-45 or ^{18}F-AV-1451 PET SUVRs of the 13 preselected brain ROIs. The LASSO variable selection method (Foubister et al., 2021; Tibshirani, 1996) was used to screen candidate risk factors in the study and the tuning parameter λ of LASSO was determined by fivefold cross-validation. The variables selected by LASSO were included in the multiple linear regression model to identify factors significantly associated with change in the SUVR. Statistical level of $p < 0.05$ was considered to be significant. SAS software (version 9.4, SAS Institute Inc.) and R glmnet package (version 4.2.4) were used for all statistical analyses.

3 | RESULTS

3.1 | Cohort characteristics

Clinical and demographic characteristics of the 255 ADNI participants are described in Table 1. A total of 127 MCI participants (31 donepezil users and 96 ChEI nonusers) received an ^{18}F-AV-45 PET scan. The average drug exposure time of donepezil users before receiving an ^{18}F-AV-45 PET scan was 5.2 ± 4.9 years. There were no

ApoE ε4	Hyperlipidemia	Donepezil	Adjusted R^2	*p*-value of regression
0.45 (0.34–0.55), **$p < 0.001$**	–	0.13 (0.02–0.25), **$p = 0.021$**	0.39	**<0.001**
0.41 (0.32–0.51), **$p < 0.001$**	–	0.16 (0.05–0.27), **$p = 0.005$**	0.39	**<0.001**
0.37 (0.28–0.45), **$p < 0.001$**	–	0.10 (0.00–0.19), **$p = 0.049$**	0.37	**<0.001**
0.36 (0.26–0.45), **$p < 0.001$**	–	0.15 (0.05–0.26), **$p = 0.004$**	0.34	**<0.001**
0.44 (0.33–0.55), **$p < 0.001$**	–	0.19 (0.06–0.32), **$p = 0.004$**	0.35	**<0.001**
0.48 (0.36–0.59), **$p < 0.001$**	−0.07 (−0.18,0.05), $p = 0.248$	0.21 (0.08–0.33), **$p = 0.002$**	0.40	**<0.001**
0.32 (0.23–0.40), **$p < 0.001$**	–	0.09 (0.00–0.19), **$p = 0.047$**	0.33	**<0.001**
0.23 (0.15–0.30), **$p < 0.001$**	–	0.09 (0.01–0.18), **$p = 0.038$**	0.24	**<0.001**

significant differences between donepezil users and ChEI nonusers in age, sex, years of education, ApoE ε4 status, and history of hypertension, hyperlipidemia, and diabetes.

A total of 128 MCI participants (23 donepezil users and 105 ChEI nonusers) received an ^{18}F-AV-1451 PET scan. The average drug exposure time of donepezil users before receiving an ^{18}F-AV-1451 PET scan was 4.8 ± 4.4 years. There were no significant differences between donepezil users and ChEI nonusers in age, sex, years of education, ApoE ε4 status, and history of hypertension, hyperlipidemia, and diabetes.

3.2 | Association between long-term donepezil use and brain regional amyloid deposition

The association between long-term donepezil use and the SUVR of ^{18}F-AV-45 PET brain ROIs assessed using multiple linear regression model with LASSO that included age, sex, years of education, ApoE ε4 status, and history of hypertension, hyperlipidemia, and diabetes is summarized in Table 2. Among the 13 preselected brain ROIs, donepezil use was associated with greater SUVR in the orbital frontal, superior frontal, lateral temporal, parietal, posterior precuneus, posterior cingulate, inferior temporal, and fusiform regions (Table 2; Figure 1a). Figure 1a shows the ROI SUVRs among donepezil users and ChEI nonusers in these brain regions with *p*-value adjusted from the final multiple regression models (Table 2). We found no significant association between donepezil use and amyloid deposition in the occipital cortex, middle temporal cortex, entorhinal cortex, amygdala, and parahippocampal gyrus (Table S1). Aside from donepezil use, ApoE ε4 status was associated with higher SUVR in all the 13 preselected brain ROIs (Tables 2 and S1). Age was significantly associated with higher SUVR in the orbital frontal, inferior temporal, lateral temporal, posterior precuneus, posterior cingulate, posterior cingulate, and the occipital cortex (Tables 2 and S1). Years of education was associated with lower SUVR in the entorhinal cortex and parahippocampal gyrus (Table S1). Sex, history of hypertension, hyperlipidemia, and diabetes were not significantly associated with SUVR of the 13 preselected brain ROIs.

Mean ^{18}F-AV-45 SUVR images of the long-term donepezil users and ChEI nonusers are presented in Figure 2a and visually demonstrate higher amyloid deposition in posterior cingulate and posterior precuneus of donepezil users compared to ChEI nonusers.

3.3 | Association between donepezil use and brain regional tau burden

The association between long-term donepezil use and brain regional ^{18}F-AV-1451 uptake is summarized in Table 3. Among the 13 preselected brain ROIs, donepezil use was significantly associated with greater SUVR in the entorhinal cortex, posterior cingulate, and parahippocampal gyrus. Figure 1b shows the SUVR of all these three brain regions among donepezil users and ChEI nonusers with *p*-values adjusted by the covariates in the final models (Table 3). In addition, the brain ROIs of middle temporal ($p = 0.064$) and amygdala ($p = 0.052$) of long-term donepezil users showed possibly greater SUVR, which need further exploration (Table S2). ApoE ε4 status was associated with greater SUVR in all the 13 preselected brain ROIs (Tables 3 and S2). Age was associated with higher SUVR only in the entorhinal cortex (Table 3). Sex was significantly associated with higher SUVR in the entorhinal cortex and parahippocampal gyrus (Table 3). Years of education, history of hypertension, hyperlipidemia, and diabetes were not significantly associated with SUVR of the 13 preselected brain ROIs.

Mean ^{18}F-AV-1451 SUVR images of the long-term donepezil users and ChEI nonusers are displayed in Figure 2b and visually demonstrate the higher tau burden in the posterior cingulate of donepezil users compared to ChEI nonusers.

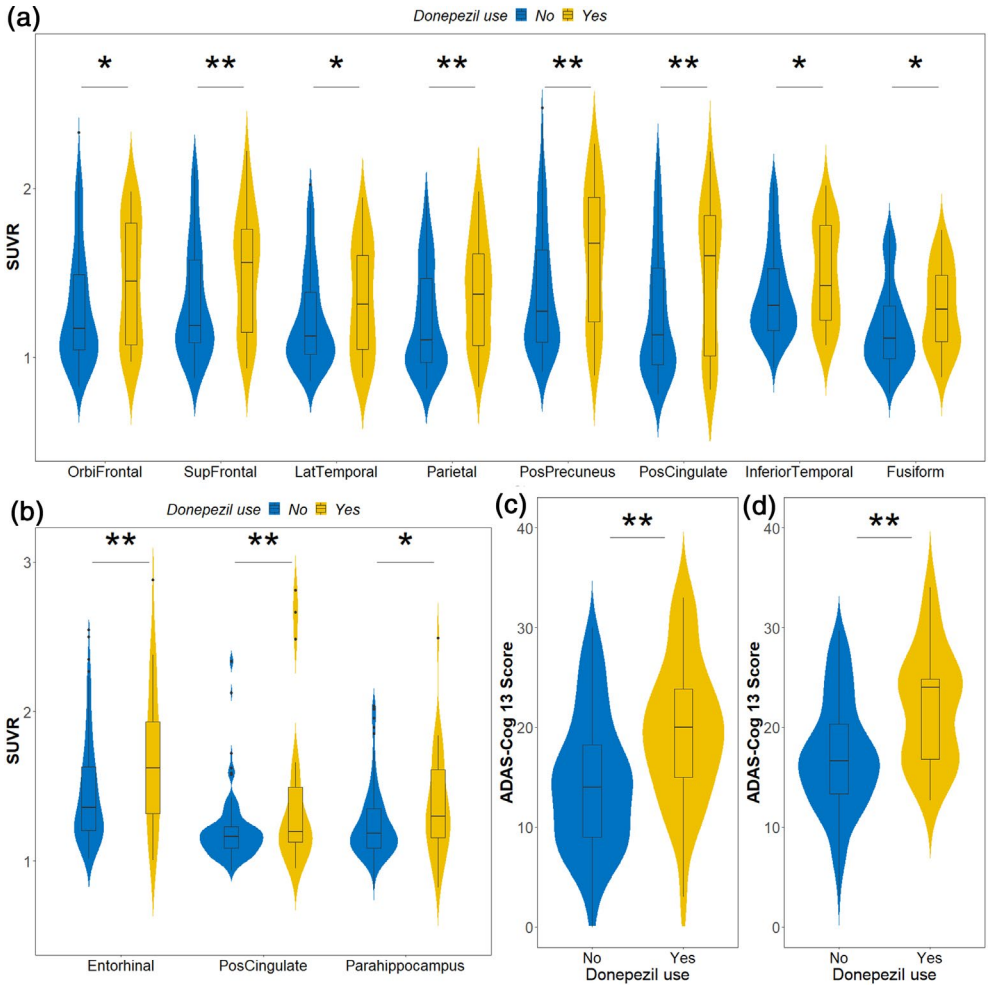

FIGURE 1 Regions with significant long-term donepezil treatment effect on ^{18}F-AV-45 SUVR (a), ^{18}F-AV-1451 SUVR (b), ADAS-cog 13 score among patients receiving ^{18}F-AV-45 scans (c) and ADAS-cog 13 score among patients receiving ^{18}F-AV-45 scans (d) after adjustment by the final multiple regression models. OrbiFrontal: orbital frontal cortex; SupFrontal: superior frontal cortex; LatTemporal: lateral temporal cortex; PosPrecuneus: posterior precuneus; PosCingulate: posterior cingulate; InferiorTemporal: inferior temporal cortex; $^{*}p < 0.05$; $^{**}p < 0.01$. Violin plots with overlaying boxplots are used to demonstrate either SUVR (A, B) or ADAS-Cog 13 score (c,d) of all study participants. The Violin plots showed the probability density of different values. The boxplots were constructed of the box and the whiskers. The box is drawn from Q1 (25th percentile) to Q3 (75th percentile) with a horizontal line drawn in the middle to denote the median. The upper whisker extends to the largest value no further than 1.5 × *IQR* from the Q3 (where *IQR* is the inter-quartile range, *IQR* = Q3−Q1). The lower whisker extends to the smallest value at most −1.5 × *IQR* from the Q1. The dots plotted individually are outliners whose values are beyond the end of whiskers (Q3 + 1.5 × *IQR* or Q1-1.5 × *IQR*)

3.4 | Donepezil use and cognitive function in patients with ^{18}F-AV-45 PET imaging

The association between long-term donepezil treatment and cognitive function for participants with ^{18}F-AV-45 PET scans is summarized in Table 4. To minimize residual confounding related to differences in AD risk factors and disease stage between donepezil users and ChEI nonusers, age, sex, years of education, ApoE ε4 status, history of hypertension, hyperlipidemia, and diabetes and ^{18}F-AV-45 PET SUVRs of the 13 preselected brain ROIs were included in the multiple regression analysis as candidate covariates. Long-term donepezil use, SUVR of posterior precuneus, and age were all associated with a greater ADAS-Cog 13 score (Table 4).

3.5 | Donepezil use and cognitive function in patients with ^{18}F-AV-1451 PET imaging

The association between long-term donepezil treatment and cognitive function for participants with ^{18}F-AV-1451 PET scans is

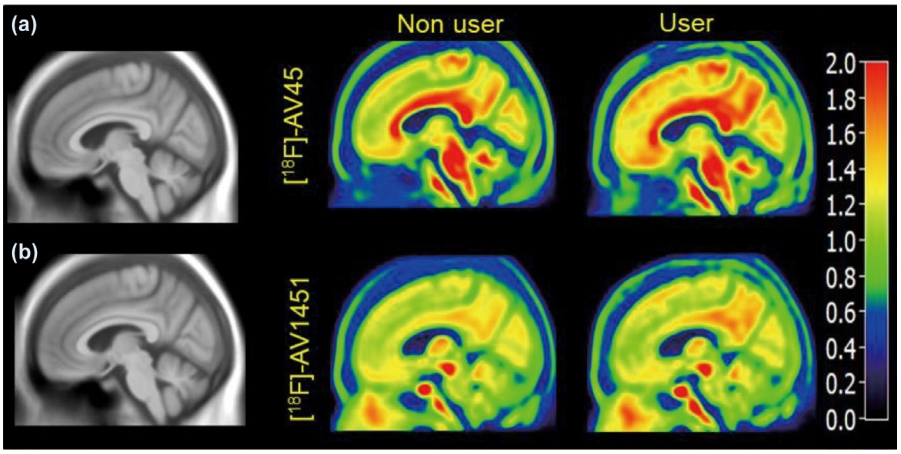

FIGURE 2 Mean ^{18}F-AV-45 (a) and ^{18}F-AV-1451 (b) SUVR images of long-term donepezil users and ChEI nonusers. Mean images were generated by computing the mean of images from long-term donepezil users and ChEI nonusers separately. The ^{18}F-AV-45 mean images (a) show a positive correlation between long-term donepezil treatment and amyloid burden in the posterior cingulate and posterior precuneus. The ^{18}F-AV-1451 mean images (b) show a positive correlation between long-term donepezil treatment and tau burden in the posterior cingulate

summarized in Table 5. Instead of adjusting by ^{18}F-AV-45 PET SUVRs, this model included a term for ^{18}F-AV-1451 PET SUVRs of the 13 preselected brain ROIs. Long-term donepezil use, SUVR of entorhinal cortex, and age were all significantly associated with a greater ADAS-Cog 13 score (Table 5).

4 | DISCUSSION

Despite its widespread use, the neuropathological consequences of long-term donepezil use remain unknown. In this study, we used multiple linear regression with LASSO to explore the relationship between long-term donepezil use and neuropathological readouts of AD progression including regional tau and amyloid burden in MCI participants. Importantly in contrast to previous studies (Babulal et al., 2020; Krell-Roesch et al., 2018; Mishra et al., 2018), we adjusted for a number of AD-associated risk factors including age, sex, ApoE ε4 status, and history of hypercholesterolemia, hypertension, and diabetes.

In contrast to previous studies which have studied the association between donepezil treatment and global Aβ and tau burden (Chalmers et al., 2009; Ishibashi et al., 2017), this is the first study to comprehensively evaluate the effect of donepezil treatment on brain regional Aβ and tau burden. Strikingly, we found a distinct pattern of tau and amyloid burden associated with long-term donepezil use. Specifically, long-term donepezil treatment was significantly associated with greater Aβ accumulation in most brain regions (Table 2) and tau burden in common tau vulnerable brain areas (Liu et al., 2019), including the entorhinal cortex, parahippocampal gyrus, and posterior cingulate cortex. Overall, these results suggest that long-term donepezil treatment may accelerate pathological Aβ and tau changes in specific brain regions.

The study used LASSO method to screen risk factors. We included age, sex, education, ApoE ε4 status, modifiable disease risk factors including history of hypertension, hyperlipidemia, and diabetes as candidate covariates. In addition to long-term donepezil treatment, the final regression model also revealed additional AD risk factors as significant predictors of regional Aβ and tau burden. Each of these risk factors is associated with specific regional patterns of brain regional Aβ and tau pathologic changes (Tables 2, 3, S1, and S2). This also helps address whether specific variables should be routinely corrected in future studies involving ^{18}F-AV-45 PET and ^{18}F-AV-1451 PET. For example, ApoE ε4 carrier status was independently associated with increased Aβ and tau deposition in all of the 13 preselected brain regions, consistent with prior studies (Gottesman et al., 2016; Liu et al., 2019; Scheinin et al., 2014), suggesting that ApoE ε4 carrier status is a major determinant of brain amyloid deposition and tau pathology (Lim et al., 2018; Shi et al., 2017). Therefore, the effect of ApoE ε4 on Aβ and tau deposition is suggested to be corrected in future quantitative AD PET studies. Age was significantly associated with greater Aβ deposition in the orbital frontal, inferior temporal, lateral temporal, posterior precuneus, posterior cingulate, posterior cingulate, and the occipital cortex, while its influence in parietal ($p = 0.058$), fusiform ($p = 0.081$), and parahippocampal gyrus ($p = 0.074$) need further investigation. Meanwhile, age was associated with tau burden only in the entorhinal cortex. This suggests that age might be more relevant to Aβ deposition than tau pathology. Female sex was significantly associated with greater tau load in the entorhinal cortex and parahippocampal gyrus, and possibly greater tau load in fusiform ($p = 0.091$) and occipital ($p = 0.072$). Moreover, sex was not significantly associated with amyloid deposition in any of the 13 preselected brain regions. Years of education was significantly associated with lower Aβ burden only in the entorhinal cortex and parahippocampal gyrus but not associated with tau burden. Future studies may consider including covariates of age, sex, and years of education only in models studying these specific brain regions. Interestingly, hyperlipidemia and hypertension were not significant independent predictors of brain regional Aβ and tau, while

diabetes was possibly associated with a higher Aβ burden in the parahippocampal gyrus ($p = 0.084$) and entorhinal cortex ($p = 0.051$). These findings may be attributed to the lower relative risk of these three common disease risk factors in promoting AD Aβ and tau pathology compared to other AD risk factors included in the final regression models. This suggests that future quantitative AD studies of the brain amyloid deposition and tau pathology may not need to correct for the effects of hypercholesterolemia and hypertension, and the effect of diabetes need further exploration. Taken together, these findings suggest that AD-related risk factors exert unique and spatially dependent effects on brain Aβ and tau deposition.

We next asked whether long-term donepezil is associated with global cognitive function after adjusting for AD-related risk factors and either brain regional Aβ or tau deposition. In models including either Aβ or tau deposition as covariates, we consistently found long-term donepezil treatment to be associated with worse global cognitive performance (Tables 4 and 5) on the ADAS-Cog 13 assessment. This is consistent with existing studies in MCI and mild AD cohorts reporting greater cognitive decline among patients with long-term ChEI exposure (Han et al., 2019; Schneider et al., 2011). Taken together, these results suggest that long-term donepezil may accelerate global cognitive decline.

Interestingly, we observed a spatially dependent pattern of amyloid and tau deposition related to cognitive function. Specifically, Aβ load in the posterior precuneus was independently and positively correlated with ADAS-Cog 13 score among participants

TABLE 3 Final multiple linear regression models showing the association between long-term donepezil use and regional tau pathology

ROI	Regression coefficient β_i (95%CI), p-value of β_i						Adjusted R^2	p-value of regression
	β_0	Age	Sex	ApoE ε4	Diabetes	Donepezil		
Posterior cingulate	1.16	–	–	0.16 (0.05–0.26), **$p = 0.006$**	–	0.18 (0.05–0.32), **$p = 0.009$**	0.10	**<0.001**
Entorhinal cortex	0.61	0.01 (0.00–0.02), **$p = 0.019$**	0.19 (0.07–0.32), **$p = 0.003$**	0.33 (0.21–0.46), **$p < 0.001$**	0.12 (−0.06–0.31), $p = 0.184$	0.21 (0.06–0.36), **$p = 0.008$**	0.27	***<0.001***
Parahippocampal	1.14	–	0.12 (0.03–0.21), **$p = 0.009$**	0.21 (0.12–0.29), **$p < 0.001$**	–	0.11 (0.00–0.22), **$p = 0.050$**	0.21	**<0.001**

Notes: All statistically significant p values are bolded.

The final multiple linear regression model used for each ROI was: SUVR(ROI) = $\beta_0 + \beta_1$ age + β_2 sex + β_3 ApoE ε4 + β_4 diabetes + β_5 donepezil. The SUVR (ROI) was measured by ^{18}F-AV-1451 tau PET. β_i = - indicates a covariate that was not included in the final model for the ROI.

Abbreviations: adjusted R^2, adjusted R square of the final multiple linear regression model; p-value of regression, p-value of the final multiple linear regression model; 95% CI: 95% confidence interval.

TABLE 4 Final multiple linear regression models showing the association between long-term donepezil use and ADAS-cog 13 in MCI patients with ^{18}F-AV-45 amyloid-β PET

Global cognition	Regression coefficient β_i (95%CI), p-value of β_i				Adjusted R^2	p-value of regression
	β_0	Posterior precuneus	Age	Donepezil		
ADAS13	−6.46	4.55 (1.55–7.55), **$p = 0.003$**	0.19 (0.05–0.34), **$p = 0.011$**	4.27 (1.58–6.96), **$p = 0.002$**	0.19	**<0.001**

Notes: All statistically significant p values are bolded.

The final multiple linear regression model used was: ADAS13 = $\beta_0 + \beta_1$ SUVR (posterior precuneus) + β_2 age + β_3 donepezil. The SUVR (ROI) was measured using ^{18}F-AV-45 amyloid PET.

Abbreviations: ADAS13, ADAS-cog 13; adjusted R^2, adjusted R square of the final multiple linear regression model; p-value of regression, p-value of the final multiple linear regression model; 95% CI, 95% confidence interval.

TABLE 5 Final multiple linear regression models showing the association between long-term donepezil use and ADAS-cog 13 in MCI patients with ^{18}F-AV-1451 tau PET

Global cognition	Regression coefficient β_i (95% CI), p-value of β_i					Adjusted R^2	p-value of regression
	β_0	Entorhinal cortex	Age	Diabetes	Donepezil		
ADAS13	−0.82	6.18 (3.88–8.49), **$p < 0.001$**	0.11 (0.01–0.22), **$p = 0.035$**	2.46 (−0.23, 5.16), $p = 0.073$	3.33 (1.01–5.65), **$p = 0.005$**	0.30	**<0.001**

Notes: All statistically significant p values are bolded.

The final multiple linear regression model used was: ADAS13 = $\beta_0 + \beta_1$ SUVR (Entorhinal cortex) + β_2 age + β_3 diabetes + β_4 donepezil. The SUVR (ROI) was measured using ^{18}F-AV-1451 tau PET.

Abbreviations: ADAS13, ADAS-cog 13; adjusted R^2: adjusted R square of the final multiple linear regression models; p-value of regression, p-value of the final multiple linear regression model; 95% CI, 95% confidence interval.

received ^{18}F-AV-45 PET scans. In contrast, tau burden in the entorhinal cortex was highly and positively associated with ADAS-Cog 13 score among participants who received ^{18}F-AV-1451 PET scans. This difference suggests that the same cognitive assessment method is associated with different patterns of pathological tau and amyloid changes, suggesting that tau pathology can progress independently of Aβ accumulation (van der Kant et al., 2019; Vogel et al., 2020).

Considering the high heterogeneity in AD, a potential limitation of the study was the relatively small sample size of the long-term donepezil MCI user group. We will continue to verify the long-term effect of donepezil on MCI users in the ongoing ADNI project with a larger sample size.

In conclusion, this study provides evidence that long-term donepezil exposure is associated with greater regional amyloid and tau burden and worse cognitive performance among individuals with MCI. These findings shed new light on the neuropathological consequences of long-term donepezil use.

DECLARATION OF TRANSPARENCY

The authors, reviewers and editors affirm that in accordance to the policies set by the *Journal of Neuroscience Research*, this manuscript presents an accurate and transparent account of the study being reported and that all critical details describing the methods and results are present.

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CONFLICT OF INTEREST

The authors have declared that no competing interest exists.

AUTHOR CONTRIBUTIONS

Conceptualization, J.L., S.H. and Y.Z.; *Methodology*, J.L., Q.G., S.H. and Y.Z.; *Resources*, S.Z.Y., C.J.Z and J.L.; *Investigation*, Q.G., C.J.Z and J.L. ; *Formal Analysis*, Q.G., and J.L.; *Software*, Q.G., C.J.Z and J.L.; *Writing - Original Draft*, Q.G. and J.L.; *Writing - Review & Editing*, M.D.P, Q.G., C.J.Z, J.L. S.H. and Y.Z.; *Visualization*, Q.G. and J.L.; *Supervision*, S.H. and Y.Z.; *Funding Acquisition*, S.H. and Y.Z.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The data sets generated during and/or analyzed during the study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

TABLE S1 Final multiple linear regression models demonstrating brain regions in which long-term donepezil use was not a significant predictor of regional amyloid-β deposition measured by ^{18}F-AV-45 amyloid-β PET SUVR

TABLE S2 Final multiple linear regression models demonstrating brain regions in which long-term donepezil use was not a significant predictor of brain regional tau pathology measured by ^{18}F-AV-1451 tau PET SUVR

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