

High Availability of the $\alpha 7$ -Nicotinic Acetylcholine Receptor in Brains of Individuals with Mild Cognitive Impairment: A Pilot Study Using ^{18}F -ASEM PET

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Emerging evidence supports a hypothesized role for the $\alpha 7$ -nicotinic acetylcholine receptor ($\alpha 7$ -nAChR) in the pathophysiology of Alzheimer's disease. ^{18}F -ASEM (3-(1,4-diazabicyclo[3.2.2]nonan-4-yl)-6- ^{18}F -fluorodibenzo[b,d]thiophene 5,5-dioxide) is a radioligand for estimating the availability of $\alpha 7$ -nAChR in the brain in vivo with PET. **Methods:** In this cross-sectional study, 14 patients with mild cognitive impairment (MCI), a prodromal stage to dementia, and 17 cognitively intact, elderly controls completed ^{18}F -ASEM PET. For each participant, binding in each region of interest was estimated using Logan graphical analysis with a metabolite-corrected arterial input function. **Results:** Higher ^{18}F -ASEM binding was observed in MCI patients than in controls across all regions, supporting higher availability of $\alpha 7$ -nAChR in MCI. ^{18}F -ASEM binding was not associated with verbal memory in this small MCI sample. **Conclusion:** These data support use of ^{18}F -ASEM PET to examine further the relationship between $\alpha 7$ -nAChR availability and MCI.

Key Words: nAChR; cholinergic; ^{18}F -ASEM; dementia; PET

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The $\alpha 7$ -nicotinic acetylcholine receptor ($\alpha 7$ -nAChR) may play a mechanistic role in the selective vulnerability of cholinergic cells to neurodegeneration in the earliest phases of Alzheimer's disease (AD) (1). The $\alpha 7$ -nAChR binds soluble 42-amino-acid β -amyloid peptide (A β 42) with picomolar affinity (2), and in AD, the A β 42– $\alpha 7$ -nAChR interaction may result in dysregulated signal transduction, compromised synaptic plasticity, and a toxic, selective effect on cholinergic cells of the basal forebrain and its projection sites (3). AD mouse models support upregulation of $\alpha 7$ -nAChR expression in the presence of A β 42, which promotes further pathologic changes (4). In AD brain tissue, high levels of

$\alpha 7$ -nAChR colocalize with intracellular A β 42 burden (5), and high $\alpha 7$ -nAChR expression was found on basal forebrain (specifically nucleus basalis) cholinergic neurons (6). Relative to control tissue, a nonsignificant trend toward higher basal forebrain $\alpha 7$ -nAChR expression was found in cases of mild cognitive impairment (MCI), a prodromal stage to dementia (6).

^{18}F -ASEM (3-(1,4-diazabicyclo[3.2.2]nonan-4-yl)-6- ^{18}F -fluorodibenzo[b,d]thiophene 5,5-dioxide) is a PET radioligand for estimating the availability of $\alpha 7$ -nAChR in the brain in vivo and has shown high specific binding in receptor blockade studies in mice (7) and baboons (8). ^{18}F -ASEM PET has been used to study the $\alpha 7$ -nAChR in healthy aging (9) and psychosis (10). In this pilot study, we used ^{18}F -ASEM PET to test for higher in vivo availability of $\alpha 7$ -nAChR in individuals with MCI than in cognitively intact, healthy controls. Associations between regional ^{18}F -ASEM binding and memory were explored.

MATERIALS AND METHODS

Human Subjects

The Johns Hopkins Institutional Review Board approved this prospective study, which was conducted under an Investigational New Drug protocol approved by the U.S. Food and Drug Administration. Participants (nonsmokers over 65 y old) provided written informed consent. Participants completed a clinical assessment, MRI examination, and comprehensive neuropsychological evaluation as previously described (9). Included was the California Verbal Learning Test (11), which is sensitive to detecting memory impairment in MCI. All MCI participants had a global clinical dementia rating (CDR) (12) of 0.5 with a sum-of-boxes score not exceeding 2.5, and impaired memory on neuropsychological testing (at least 1 SD below normal performance on a memory test). Control participants were in stable health, with a global CDR of 0 (normal). Exclusion criteria included a global CDR of more than 0.5, a decline in activities of daily living, nicotine use in the past 6 mo, a neurological condition other than MCI, substance abuse, current use of medication with potential to affect radiotracer binding (i.e., acetylcholinesterase inhibitors and anticholinergics), or a contraindication to imaging.

The presence of an apolipoprotein E (APOE) $\epsilon 4$ allele is associated with cerebral amyloid burden (13). We classified participants as either an APOE $\epsilon 4$ carrier (presence of ≥ 1 $\epsilon 4$ allele) or an APOE $\epsilon 4$ noncarrier, using previously published genotyping methods (9).

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Brain Imaging

¹⁸F-ASEM PET and MRI data were acquired, and PMOD (version 3.7) was used for image processing as previously described (9). MRI volumetric segmentation was conducted with the FreeSurfer software suite (<http://surfer.nmr.mgh.harvard.edu/>) or MRICloud (www.mricloud.org; for basal forebrain only). The regions of interest included cortical (frontal, parietal, temporal, occipital, cingulate, and cerebellar) and subcortical (hippocampus, thalamus, striatum, and basal forebrain) regions. Total intracranial volume was also estimated using FreeSurfer for comparison of regional atrophy between the 2 groups while accounting for individual variability in head size. Regional volume normalized to intracranial volume is referred to as regional volume ratio.

The kinetics of ¹⁸F-ASEM were modeled using Logan graphical analysis (14) with a metabolite-corrected arterial input function obtained from 90 min of dynamic data, generating a regional ¹⁸F-ASEM total distribution volume, V_T (15). To address the effect of atrophy on ¹⁸F-ASEM binding in the brains of this elderly study population, we report regional V_T values that were derived from PET data after partial-volume correction (PVC) using the algorithm of Müller-Gärtner et al. (16). V_T estimates from images without PVC were secondary binding outcomes.

Statistical Analysis

Differences in ¹⁸F-ASEM V_T between the control and MCI groups were tested using a linear mixed-effects regression model to accommodate within-subject assessment of brain regions that are associated to different degrees. Primary predictors included group, an index variable for brain region, and the 2-way interaction. Significance was set at a P value of less than 0.05 for this single mixed-effects regression model analysis. Pearson partial correlations were conducted to explore relationships within the MCI group between ¹⁸F-ASEM binding and performance on California Verbal Learning Test short- and long-delay free recall. Age was included in all models as a covariate because age may affect ¹⁸F-ASEM binding (9). When group differences emerged, subsequent models were run with the adjustment for not only age but also for race, sex, and *APOE* $\epsilon 4$ carrier status. Because there were 2 primary memory outcomes, the threshold for

significance was set at a P value of less than 0.025 ($=0.05/2$) for examining these correlations.

RESULTS

Participants

Clinical characteristics, *APOE* $\epsilon 4$ carrier status, and relevant imaging parameters were similar between the control ($n = 17$) and MCI ($n = 14$) groups (Table 1). After age adjustment, patients with MCI had higher scores than controls on the sum-of-boxes CDR ($P = 0.001$) and lower scores on the 2 California Verbal Learning Test outcomes (each $P < 0.025$).

Imaging

In age-adjusted analyses, there was a near-significant group \times region interaction ($F_{9,28} = 2.21$, $P = 0.05$) indicating that there were group differences in some but not all MRI-based segmentation regional volumes. Specifically, the MCI group had smaller hippocampal ($P = 0.001$) and thalamic ($P = 0.04$) volumes than controls (Supplemental Table 1; supplemental materials are available at <http://jnm.snmjournals.org>). The same pattern of differences was seen in regional volume ratios after age adjustment.

The metabolism of ¹⁸F-ASEM over the scan duration did not differ between groups at any time point. At 10, 45, and 90 min after injection, mean parent fractions were $69\% \pm 7\%$, $28\% \pm 9\%$, and $14\% \pm 5\%$, respectively, among the MCI group and $68\% \pm 6\%$, $29\% \pm 8\%$, and $15\% \pm 4\%$, respectively, among the controls. In age-adjusted analyses using images after PVC, the MCI group had a higher ¹⁸F-ASEM V_T (estimated mean, 31.51; SE, 1.14) than did controls (estimated M, 27.23; SE, 1.04; $F_{1,28} = 7.02$, $P = 0.01$; Cohen's $d = 0.999$; 95% confidence interval, 0.25–1.75), with the pattern of group differences similar across all regions ($F_{9,28} = 1.37$, $P = 0.25$; Fig. 1; Supplemental Table 2). Rerunning the model after further adjusting for race, sex, and *APOE* $\epsilon 4$ carrier

TABLE 1
Clinical Characteristics and PET Parameters Among Healthy Controls and Patients with MCI

Characteristic	Control ($n = 17$)	MCI ($n = 14$)	P
Age (y)	76.4 \pm 6.2	80.5 \pm 5.5	0.06
Sex (male)	9 (53%)	7 (50%)	0.88
Race (African American; Caucasian; Asian)	4 (24%); 12 (71%); 1 (6%)	7 (50%); 7 (50%); 0 (0%)	0.24
Education (y)	17.1 \pm 3.3	15.7 \pm 4.3	0.35
Body mass index	26.5 \pm 3.0	26.7 \pm 3.2	0.92
Genetic vulnerability, <i>APOE</i> $\epsilon 4$ carrier (yes)	3 (18%)	5 (36%)	0.28
Cognitive performance			
Sum-of-boxes CDR	0.0 \pm 0.1	1.1 \pm 0.7	<0.001
CVLT short-delay free recall	11.2 \pm 2.9	7.9 \pm 4.0	0.02
CVLT long-delay free recall	11.8 \pm 2.7	7.6 \pm 5.0	0.02
PET parameters			
Molar activity (GBq/ μ mol)	3,878.1 \pm 2,614.6	3,327.1 \pm 2,133.8	0.52
Injected dose of radioactivity (MBq)	516.2 \pm 22.2	517.2 \pm 17.7	0.89
Injected mass (μ g)	0.08 \pm 0.06	0.11 \pm 0.12	0.43

CVLT = California Verbal Learning Test.

P values are from comparison using Student t test or χ^2 test as appropriate except for comparison of cognitive performance, which was assessed using analysis of covariance with age as covariate. Qualitative data are expressed as numbers followed by percentages in parentheses; continuous data are expressed as mean \pm SD.

status resulted in the same pattern of higher ^{18}F -ASEM V_T in MCI patients than in controls ($F_{1,24} = 11.03$, $P = 0.003$).

Among secondary ^{18}F -ASEM binding outcomes (Supplemental Table 3), a similar but nonsignificant binding pattern was observed using V_T derived from data without PVC and age adjustment ($F_{1,28} = 3.81$, $P = 0.06$). After further adjusting for race, sex, and *APOE* $\epsilon 4$ carrier status, the group difference was significant ($F_{1,24} = 9.06$, $P = 0.006$).

Among patients with MCI, there were no associations between regional ^{18}F -ASEM V_T and California Verbal Learning Test performance (Supplemental Table 4).

DISCUSSION

Our ^{18}F -ASEM PET data are consistent with higher availability of the $\alpha 7$ -nAChR in MCI patients than in healthy controls across multiple brain regions. These pilot results align with postmortem studies reporting higher $\alpha 7$ -nAChR levels in early stages of AD (6), as well as with animal models of AD (4,17). If validated as a mechanistically linked biomarker, high $\alpha 7$ -nAChR availability may prove useful for earlier detection and therapeutic intervention in AD (18,19).

We acknowledge limitations in this study. First, V_T reflects specifically bound and nondisplaceable (nonspecifically bound or free) radiotracer. Human ^{18}F -ASEM PET data do not support a brain region without $\alpha 7$ -nAChR that could be used as reference tissue for reporting the outcome of specifically bound (relative to nondisplaceable) ^{18}F -ASEM. This widespread $\alpha 7$ -nAChR distribution in human brain is similar to that reported in rhesus monkeys and differs from rodent brain (20). V_T was derived from images after PVC that adjusts the PET signal for regional brain atrophy seen in neurodegeneration. Consistent with published MCI work and supporting use of PVC, we saw smaller hippocampal and thalamic

volumes in MCI patients than in controls after age adjustment. However, a risk of using PVC is false elevation of regional V_T estimates due to overcompensation by the method. In this study, model analysis using V_T from images without PVC produced a similar, albeit nonsignificant, pattern of higher binding in MCI patients than in controls after age adjustment, which achieved significance after further adjustment for other factors (sex, race, and *APOE* $\epsilon 4$ carrier status). Second, whereas these findings suggest a role for the $\alpha 7$ -nAChR in MCI, the cross-sectional study design and lack of amyloid markers in our pilot population limit the ability to assess causality or specificity to AD-mediated MCI (as opposed to MCI from non-AD pathology). The lack of association between ^{18}F -ASEM V_T and verbal memory impairment in this study may be due to possible inclusion of MCI participants with non-AD disease. Furthermore, here we focused on brain tissue, but the $\alpha 7$ -nAChR in cerebral vasculature may promote cerebral amyloid angiopathy (21), which is common in AD and linked to impaired memory. Complementary animal studies that manipulate $\alpha 7$ -nAChR availability or activity should be pursued to evaluate causal, mechanistic relationships between the $\alpha 7$ -nAChR and AD pathophysiology. Future studies designed to test the relationships between ^{18}F -ASEM binding and neuropsychological performance in larger clinical samples are needed.

CONCLUSION

Our ^{18}F -ASEM PET data support a higher availability of the $\alpha 7$ -nAChR across several brain regions in individuals with MCI than in healthy individuals.

DISCLOSURE

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KEY POINTS

QUESTION: Is the availability of the $\alpha 7$ -nicotinic acetylcholine receptor ($\alpha 7$ -nAChR) altered in brains of individuals with mild cognitive impairment (MCI)?

PERTINENT FINDINGS: In this cross-sectional study using PET, regional binding of ^{18}F -ASEM that targets the $\alpha 7$ -nAChR was compared between patients MCI and cognitively intact, elderly controls. Higher ^{18}F -ASEM binding was observed in MCI compared with controls across all brain regions.

IMPLICATIONS FOR PATIENT CARE: ^{18}F -ASEM is a promising radiotracer to test further the relationship between the availability of the $\alpha 7$ -nAChR and MCI in vivo with PET.

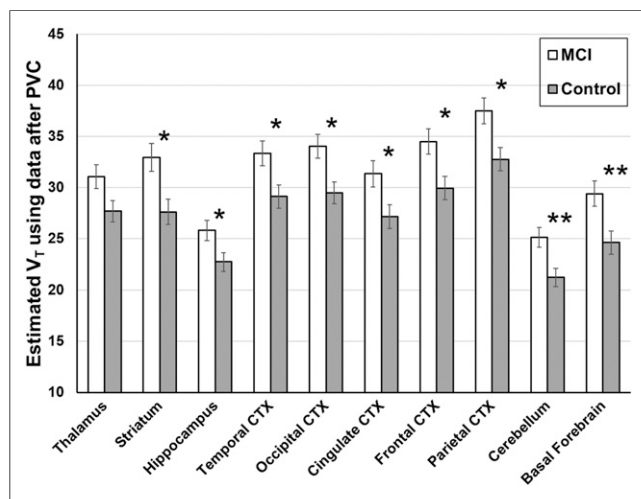


FIGURE 1. Higher ^{18}F -ASEM regional V_T was found in 14 participants with MCI than in 17 cognitively intact healthy control participants, after accounting for age. Box plot diagram of ^{18}F -ASEM V_T was derived from images after PVC across 10 regions of interest. A linear mixed-effects regression model adjusting for age and brain region demonstrated higher V_T in MCI patients than in controls. In secondary analyses, a regional difference for each region except thalamus was found between MCI patients and controls using individual linear regression models fit for region of interest and controlling for age (* $P < 0.05$; ** $P < 0.01$). These differences in individual regions did not remain significant after Bonferroni adjustment for 10 regions tested, which required $P < 0.005$. V_T is in units of mL cm^{-3} . CTX = cortex.

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