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Reduced medial temporal lobe *N*-acetylaspartate in cognitively impaired but nondemented patients

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Abstract

Background—*N*-acetylaspartate (NAA) in the medial temporal lobe (MTL) and parietal lobe gray matter (GM) is diminished in Alzheimer disease (AD). Because NAA is considered a marker of neuronal integrity, reduced medial temporal and parietal lobe NAA could be an early indication of dementia-related pathology in elderly individuals.

Objectives—1) To determine whether cognitively impaired but nondemented (CIND) elderly individuals exhibit a similar pattern of reduced medial temporal and parietal lobe NAA as AD patients. 2) To compare regional NAA patterns, hippocampal and neocortical gray matter (GM) volumes in CIND patients who remained cognitively stable and those who became demented over 3.6 years of follow-up. 3) To examine the relationship between memory performance, medial temporal lobe NAA, and hippocampal volume.

Methods—Seventeen CIND, 24 AD, and 24 cognitively normal subjects were studied using MRSI and MRI.

Results—Relative to controls, CIND patients had reduced MTL NAA (19 to 21%, $p = 0.005$), hippocampal (11 to 14%, $p \leq 0.04$), and neocortical GM (5%, $p = 0.05$) volumes. CIND patients who later became demented had less MTL NAA (26%, $p = 0.01$), hippocampal (17 to 23%, $p \leq 0.05$), and neocortical GM (13%, $p = 0.02$) volumes than controls, but there were no significant differences between stable CIND patients and controls. MTL NAA in combination with hippocampal volume improved discrimination of CIND and controls over hippocampal volume alone. In AD and CIND patients, decreased MTL NAA correlated significantly with impaired memory performance.

Conclusion—Reduced medial temporal lobe *N*-acetylaspartate, together with reduced hippocampal and neocortical gray matter volumes, may be early indications of dementia-related pathology in subjects at high risk for developing dementia.

Alzheimer disease (AD) and vascular dementia are both preceded by a preclinical phase during which cognitive deficits are detectable.¹ For this reason, numerous studies have used ¹H MR

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spectroscopy (MRS), which affords the opportunity to visualize and quantify metabolites and amino acids in the living human brain, with the aim of discovering early markers of dementia. Many MRS studies have reported elevations in myoinositol (mI) concentration to be a reliable and specific marker of early dementia.² Some MRS studies have also found decreased concentrations of *N*-acetylaspartate (NAA) in patients with age-associated memory impairment (AAMI)³ or mild cognitive impairment (MCI)⁴ relative to controls. For example, lower mean NAA has been found in the temporo-parietal region of AAMI patients relative to controls.⁵ Lower NAA/Creatine (Cr) and NAA/mI ratios have also been noted in the posterior cingulate of MCI patients relative to controls. However, this finding was significant in some⁶ but not all⁷ MRS data obtained on a 1.5 T scanner and was not significant in MRS data obtained from a 3 T scanner.⁶ Recently lower NAA/H₂O ratios have been reported in the left MTL of MCI patients relative to controls.⁸

We have previously shown that AD is characterized by diminished NAA in the MTL and parietal gray matter.⁹ Because NAA is considered a marker of neuronal integrity due to its localization in neurons and axons of the adult brain, a selective reduction of NAA in the MTL and parietal lobe GM may be early indicators of dementia-related pathology in elderly individuals who are at risk for developing dementia. We sought to examine whether cognitively impaired but nondemented (CIND) elderly adults who are at increased risk for developing dementia present with a similar pattern of diminished NAA in the MTL and parietal lobe GM as that previously seen in AD patients. Rates of hippocampal atrophy have been reported to match the change in cognitive status over time in elderly individuals who lie along the cognitive continuum from normal aging to MCI to AD.¹⁰ Another study found that baseline hippocampal volumes can predict subsequent conversion to AD in older MCI patients.¹¹ Therefore, we compared regional NAA patterns, hippocampal, and neocortical GM volumes in CIND patients who remained cognitively stable and those who converted to dementia over a follow-up period of approximately 3.6 years. Hippocampal volume has been shown to predict declarative memory performance across the spectrum of normal aging to AD.¹² MTL metabolic alterations have been shown to correlate with cognitive decline and verbal memory loss in AD patients.^{13,14} Thus, we investigated the relationship between hippocampal volume, MTL NAA concentration, and declarative memory performance in AD and CIND patients.

Methods

Subjects

Seventeen patients (mean age: 75.4 ± 6.8 years) with cognitive impairment not meeting criteria for dementia (i.e., CIND) and 24 patients (mean age: 74.8 ± 6.9 years) with a clinical diagnosis of probable ($n = 20$) or possible ($n = 4$) AD according to the National Institute of Neurologic and Communicative Disorders and Stroke–AD and Related Disorders Association (NINCDS-ADRDA) criteria¹⁵ were recruited from Alzheimer's centers at the University of California at San Francisco (UCSF) and Davis (UCD). All of the CIND patients had a clinical dementia rating (CDR) of 0.5 and scored at least 1.5 SD below the age- and education-adjusted mean on at least one (in many cases more than one) measure of verbal declarative memory. Twelve CIND patients had clinical follow-ups over a period of 3.6 ± 1.7 years (range: 1 to 7 years). During this time, the clinical classification of six patients remained unchanged while six patients became demented (two probable AD, two mixed dementia, two vascular dementia) after an average of 2.8 ± 0.8 years following their initial MRI and MRSI examination. The patients who subsequently converted to dementia were diagnosed at a multidisciplinary case conference using the NINCDS-ADRDA diagnostic criteria for AD¹⁶ and the California AD Diagnostic and Treatment Center (ADDTC) criteria for ischemic vascular dementia.¹⁷ Twenty-four cognitively normal subjects (mean age: 76.0 ± 6.3 years) were recruited from the community and received standard neuropsychological examinations at the same Alzheimer's

centers. AD patients had Mini-Mental State Examination (MMSE)¹⁸ scores that ranged from 4 to 26. CIND patients had MMSE scores that ranged from 20 to 30. Control subjects had cognitive test scores within the normal age and education-adjusted range. No subject had a clinical history of psychiatric illness (other than dementia), epilepsy, hypertension, diabetes, major heart disease, head trauma, or alcoholism. Behavioral performance on the Memory Assessment Scale (MAS) word list learning test¹⁹ was available for 9 AD patients, 14 CIND patients, and 9 control subjects.

A neuroradiologist evaluated all of the MRI scans, especially for the presence of vascular pathology. No subject included in this study had more than one lacunar infarct and none had major vascular pathologies, other than white matter T2 signal hyperintensities (WMH). MRI and MRSI data from most of the AD (n = 23) and control subjects (n = 20) have previously been reported.⁹ Informed consent, approved by the committees for human research at UCSF, UCD, and the San Francisco Veterans Affairs Medical Center, was obtained from each subject or his or her legal guardian prior to participation in the study.

MRI and ¹H MRSI acquisition

MRI and ¹H MRSI data were obtained on a standard 1.5 Tesla Siemens Vision System (Siemens, Islen, NJ). Acquisition parameters are largely similar to those described previously.²⁰ A point-resolved spectroscopy (PRESS) ¹H MRSI²¹ sequence (repetition time [TR]/echo time [TE] = 1,800/135 msec; in-plane resolution 8.5 × 8.5 mm²; 15-mm thick section, 1.1 mL nominal voxel size) was used to acquire water-suppressed ¹H MR spectra simultaneously from the left and right hippocampal regions, as previously reported.²² After B₀-field homogeneity was restored across the brain using an automated shimming routine, water-suppressed ¹H MR spectra were acquired from two axial-oblique 15-mm-thick sections of the frontal and parietal brain regions using a multislice ¹H MRSI sequence (TR/TE = 1,800/135 msec). The MRSI slices covered large areas of the left and right frontal and parietal lobes with little inclusion of the occipital and temporal lobes and subcortical regions. The nominal MRSI voxel size was approximately 0.9 mL.

MRI volume measurements

Volumes of the left and right hippocampus were measured by manually drawing the boundaries of this structure on the coronal-oblique T1-weighted MR images, as described previously.²³ Tissue segmentation of the MRI data into gray matter (GM), white matter (WM), and CSF was achieved automatically with software developed in-house.²⁴ Additional operator-assisted segmentation classified the GM further into cortical and subcortical GM, the WM into normal WM and WM lesions, and the CSF into sulcal and ventricular CSF. To account for variations in head size among subjects, the segmentation and hippocampal volume results were normalized to the total intracranial volume (TIV) according to the following formula:

$$V_{\text{norm}}^i = V_o^i * \text{TIV}^m / \text{TIV}^i$$

where V_{norm}^i is the normalized volume, V_o^i is the absolute volume, and $\text{TIV}^m/\text{TIV}^i$ is an index of the subject's TIV relative to the overall mean TIV for the entire group (i.e., AD, CIND, and controls combined).

Spectral processing and MRI-MRSI coanalysis

Both PRESS and multislice ¹H MRSI data were processed and peak areas of NAA, Cr, and choline (Cho) were estimated using the same fully automated spectral fitting software developed in-house.²⁵ Processing parameters have been previously described in detail.²⁰ Briefly, PRESS MRSI voxels in the right and left hippocampus that provided simultaneously large amounts of hippocampal tissue and high spectral quality (i.e., 2Hz < linewidth < 10 Hz)

were selected automatically. These voxels also extended into adjacent brain regions, such as the entorhinal cortex (ERC), and included a mixture of GM and WM. The PRESS voxels were corrected for CSF by referencing the metabolite intensities to gray and white matter content within each voxel. To avoid computing artificially high metabolite values, only PRESS voxels with at least 75% brain tissue were included in the analyses. For multislice ^1H MRSI data, metabolite contributions from GM and WM were separated by regressing metabolite intensity variations against variations in GM, WM, and CSF tissue composition across MRSI voxels, as previously described.²⁰ The regression coefficients represent metabolite intensities per volume GM or WM tissue, which is synonymous with concentration (in arbitrary units) in this context. These are the values reported here. However, metabolite concentrations (denoted [NAA], [Cr], [Cho]) of the PRESS and multislice ^1H MRSI cannot be directly compared with absolute concentrations in units of mmol/L because T1 and T2 were not measured and intensity was not calibrated. Spurious contributions to the metabolite signal from other tissue types and regions (e.g., subcortical GM or occipital lobe) were accounted for by screening out MRSI voxels from those regions.

Statistical analysis

Regional differences in metabolite concentrations between CIND, AD, and control subjects were tested using multivariate analysis of covariance (MANCOVA), with age and WMH as covariates. In addition, variations in the amount of GM and WM in PRESS voxels were included as covariates in analyses of the PRESS MRSI data to account for differences of metabolite concentration between these two tissue types. Metabolite concentrations were the dependent variables while brain region and diagnosis were the independent variables. Contrasts were used to perform between-group comparisons. To compare PRESS, multislice MRSI data, and volumetric MRI data without needing to account for experimental differences between the techniques, metabolite concentrations and volumes were transformed into Z-scores. Spearman's rank order correlation was used to analyze the relationship between right and left MLT NAA and right and left hippocampal volume across the entire group, across the CIND and AD groups combined, and within the CIND and AD groups individually. The [H9251]-level was 0.05 for all comparisons. We also used Spearman's rank order correlation to analyze the relationship between hippocampal volume, MTL NAA, and memory performance. Because we had no a priori hypotheses about laterality, right and left hippocampal volumes and right and left MTL NAA were combined for these correlations. Moreover, in light of the small number of subjects with psychometric data, we excluded control subjects and combined the AD and CIND patients' data together for the analysis. This resulted in six comparisons: MTL NAA, hippocampal volume, and three conditions of the MAS word list-learning test (i.e., list-learning, short-delay recall, and long-delay recall). For these correlations, the [H9251]-level was raised to $0.05/6 = 0.008$ to reduce the probability of finding by-chance significance.

Results

Table 1 lists the demographic and clinical data of the study groups. The groups were similar with regard to age and amount of WMH ($p > 0.8$ for both). A χ^2 test of independence revealed no differences in the male-to-female ratios among the study groups ($\chi^2 = 1.74$, $df = 2$, $p = 0.42$). As expected, mean MMSE scores were lower in AD patients than controls ($p < 0.0001$) and CIND patients ($p < 0.0001$). However, there were no significant MMSE score differences between controls and CIND patients.

Table 2 lists the MRI derived volumes for the hippocampus and GM. After accounting for age and WMH, there were group differences in hippocampal (left: $F_{2,61} = 26.02$; right: $F_{2,63} = 13.95$; $p < 0.0001$ for both) and neocortical GM ($F_{2,64} = 3.43$; $p = 0.039$) volumes. Between-group comparisons revealed that AD patients had less hippocampal (30% in the left; 22% in the right, $p < 0.001$ for both) and neocortical GM (6%, $p = 0.017$) volumes than controls. AD

patients also had smaller hippocampi (23% in the left, $p < 0.0001$; 14% in the right, $p = 0.008$) than CIND patients. There were no significant neocortical GM volume differences between CIND and AD patients. CIND patients had less hippocampal (14% in the left; 11% in the right, $p = 0.04$, $p = 0.007$) and neocortical GM (5%, $p = 0.05$) volumes than controls. Dichotomizing CIND patients revealed that converters had less hippocampal (23% in the left, $p = 0.003$; 17% in the right, $p = 0.051$) and neocortical GM (13%, $p = 0.002$) volumes than controls. In contrast, there were no significant differences between stable CIND patients and controls. Relative to stable CIND patients, CIND patients who later converted to dementia had 22% less hippocampal volume in the left hemisphere ($p = 0.02$) and 11% less neocortical GM ($p = 0.045$) volume.

The figure shows representative proton spectra obtained from the left MTL region, NAA images, and corresponding structural MRI in a healthy control subject, a CIND patient, and an AD patient. Table 3 lists MTL NAA concentration for healthy controls, AD, and CIND patients. After accounting for age, WMH, and variations in the amount of GM/WM in the PRESS voxels, there were group MTL [NAA] differences in both hemispheres (left: $F_{2,63} = 6.02$; $p = 0.004$; right: $F_{2,64} = 9.09$; $p < 0.0001$). Between-group comparisons revealed that AD (20% in the left, $p = 0.004$; 25% in the right, $p < 0.0001$) and CIND patients (21% in the left; 19% in the right, $p = 0.005$ for both) both had less MTL [NAA] than controls. There were no significant MTL [NAA] differences between CIND and AD patients. Dichotomizing CIND patients revealed less MTL [NAA] in CIND converters than controls (26% in the left, $p = 0.01$; 18% in the right, $p = 0.09$), but no significant differences between stable CIND patients and controls. None of the MTL [NAA] differences between CIND-converters and stable CIND patients reached significance.

In order to provide comparisons to other studies in the field, we also list NAA/Cr ratios in table 3. After accounting for age, WMH, and variations in the amount of GM/WM in the PRESS voxels, analysis of NAA/Cr ratios revealed a main effect of group in the left MTL ($F_{2,63} = 3.93$, $p = 0.025$). Pairwise contrasts revealed left MTL NAA/Cr differences between AD patients and controls ($p = 0.009$), a trend for MTL NAA/Cr differences between CIND patients and controls ($p = 0.07$), but no difference between CIND and AD patients or between CIND-converters and stable CIND patients. Relative to the metabolite ratios, absolute concentrations of MTL NAA showed greater significance and larger effect sizes when comparing AD and CIND vs controls (in left MTL: absolute concentration effect size: 0.9; ratio effect size: 0.6 for both). This demonstrates the value of determining absolute metabolite concentrations.

Table 4 lists NAA concentrations in the parietal and frontal lobe GM of control subjects, AD, and CIND patients. After accounting for age, WMH, and variations in the amount of GM/WM in the MRSI voxels, there was a main group effect for left parietal lobe GM [NAA] ($F_{2,64} = 4.88$; $p = 0.01$) and trends toward a group effect for right parietal lobe GM [NAA] ($F_{2,64} = 2.74$; $p = 0.07$). Between-group comparisons revealed that AD patients had less parietal GM [NAA] (24% in the left, $p = 0.008$; 19% in the right, $p = 0.07$) than controls, but no significant parietal GM [NAA] differences between CIND patients and controls or between CIND and AD patients. Comparisons of parietal GM [NAA] between CIND-converters and stable CIND patients yielded no significant differences, in contrast to neocortical GM volume. There were also no significant group differences for [Cho] and [Cr] in parietal, or frontal lobe GM; however, the small sample size may have reduced statistical power and may account for our failure to detect significance.

Because there were different numbers of men and women in the AD and CIND groups, we examined the extent to which sex contributed to regional [NAA] differences. This analysis revealed trends for male AD ($p = 0.09$) and male CIND patients ($p = 0.05$) to have lower MTL [NAA] in the left hemisphere than female patients. Otherwise, there were no significant effects

of sex on [NAA] in any of the other brain regions examined. We also tested for hemispheric differences in [NAA] across all subjects and found no effects of hemisphere and no hemisphere by group interactions.

Spearman's rank correlations (two-tailed) were used to examine the relationship between right and left MLT [NAA] and right and left hippocampal volume across the entire group, across CIND and AD patients combined, and within the CIND and AD groups individually. When all subjects were analyzed together, MLT [NAA] correlated with hippocampal volume (left: $r = 0.32, p = 0.013$; right: $r = 0.36, p = 0.042$; both hemispheres: $r = 0.34, p = 0.008$). However, there were no significant correlations between MTL [NAA] and hippocampal volume when the CIND and AD groups were analyzed together or individually.

To compare regional [NAA] differences with each other and with MRI-derived volume data, we transformed MRS and MRI volume data into Z-scores. This analysis revealed no differences in regional [NAA] Z-scores in either the AD ($F_{2,46} = 2.03, p > 0.14$) or CIND group ($F_{2,30} = 0.45, p > 0.64$). There were also no differences between regional [NAA] and MRI volume Z-scores in CIND patients ($F_{4,64} = 1.57, p = 0.22$); however, there were differences in AD patients ($F_{4,88} = 10.70, p < 0.0001$). Pairwise comparisons revealed that this was due to the fact that hippocampal volume reductions in AD patients were greater than neo-cortical GM volume reductions ($p < 0.0001$) and [NAA] changes in frontal lobe GM ($p < 0.0001$), parietal lobe GM ($p = 0.005$), and the MTL ($p = 0.001$).

We next tested the extent to which MTL [NAA] measurements combined with MRI volume measurements improve discrimination between CIND and controls. Table 5 lists the sensitivity, specificity, and overall correct classification of CIND and control subjects for those combinations of MRSI and MRI measures that made significant contributions to the classification. Also listed are the areas under the curve from a receiver operator characteristic (ROC) analysis. Prediction of group membership by hippocampal volume alone was significant ($p = 0.015$), yielding a ROC area under the curve of 0.78. Adding MTL [NAA] improved classification ($p = 0.025$) and increased the area under the ROC curve to 0.84. However, additional contributions from neocortical GM volume were not significant.

Finally, we examined the relationship between MTL [NAA], MRI-derived hippocampal volumes, and memory performance. When the AD and CIND patients' psychometric data were considered together, decreased MTL [NAA] was correlated with the poor performance on all three conditions of the MAS word-list learning test (list learning: $r = 0.63, p = 0.002$; short-delay recall: $r = 0.58, p = 0.004$; long delay recall: $r = 0.59, p = 0.004$). Decreased hippocampal volume was correlated with the short-delay recall condition of the MAS word-list learning test ($r = 0.56, p = 0.007$).

Discussion

The major findings of this study were 1) NAA concentration in the MTL, encompassing the hippocampus and ERC, hippocampal, and neocortical GM volumes were all significantly reduced in CIND patients relative to control subjects. 2) CIND patients who later became demented had lower MTL NAA, smaller hippocampi, and less neocortical GM volume than controls. In contrast, there were no significant differences between stable CIND patients and controls. 3) Decreased MTL NAA in AD and CIND patients correlated significantly with impaired declarative memory performance. These results suggest that MTL NAA reduction is biologically significant and, together with decreased hippocampal and neocortical GM volumes, may be an early indication of dementia-related pathology in elderly individuals who are at risk for developing dementia.

The first major finding of this study is that MTL NAA, together with hippocampal and neocortical GM volumes, was significantly reduced in CIND patients relative to controls. Several studies have reported reduced hippocampal^{23,26,27} and neocortical GM volumes²³ in subjects at high risk for developing dementia (e.g., AAMI, MCI, or CIND patients) relative to controls. Many investigators have also noted elevations of myoinositol in patients with MCI relative to controls.² However, there have been relatively few reports of spectroscopic abnormalities involving NAA in this subject population. Moreover, no study has used MRS data together with quantitative hippocampal volumetry and measurements of neocortical GM volumes with tissue segmentation.

A previous single voxel MRS study reported lower NAA in the temporo-parietal region of six AAMI subjects relative to six controls.⁵ Another single-voxel MRS study found increased ml/Cr ratio in the paratrigoal white matter in MCI patients relative to controls, but no significant NAA/Cr differences.²⁸ This is consistent with our previous report of no significant white matter NAA differences in AD and subcortical ischemic vascular dementia patients relative to controls.²⁹ Although the lack of white matter NAA alterations may, at first glance, appear to undercut the neuronal loss/dysfunction hypothesis, there are at least two possible explanations for this observation. First, NAA changes in AD and CIND/MCI patients may be confined to specific white matter tracts or projections that occupy only a small volume fraction and therefore may have remained undetected. Second, NAA alterations may primarily indicate damage to neuronal cell bodies and their processes, including diminished mitochondrial metabolism. One group has reported lower NAA/Cr and NAA/ml ratios the posterior cingulate of MCI patients relative to controls.⁶ However, these results were only significant in data obtained on a 1.5 T scanner and not in data obtained from a 3 T scanner.⁶ This is unexpected given that spectral resolution and signal-to-noise ratios generally improve with higher magnetic field strengths.³⁰ Furthermore, this group did not report any significant NAA/Cr or NAA/ml differences between MCI patients and controls in an earlier publication using identical scanning parameters on a 1.5 T scanner.⁷ Recently, lower NAA/H₂O ratios have been reported in the left MTL of MCI patients relative to controls.⁸ Although that MRS study examined a different region of the MTL (amygdala, anterior half of the hippocampus, and parts of the underlying subiculum) with a larger voxel (7 to 8 cm³) than the current study (1.2 cm³ voxel in the posterior portion of the hippocampus and the ERC), the results of both studies indicate that MTL NAA is reduced in elderly individuals at increased risk for developing dementia. Because NAA is diminished in conditions associated with neuronal loss, such as cerebral infarction,³¹ together, these findings suggest that MTL NAA reduction may be an early marker of dementia-related pathology. However, reduced NAA has also been noted in potentially intact neurons with impaired function, such as in an acute MS plaque.³¹ Moreover, reversible NAA loss following treatment has been reported in amyotrophic lateral sclerosis.³² Considered in this context, MTL NAA could also be potentially useful in monitoring the restorative effects of future treatments for dementia.

The second major finding of this study is that CIND patients who later became demented had lower MTL NAA, smaller hippocampi, and reduced neocortical GM volumes relative to control subjects. In contrast, there were no significant differences between controls and CIND patients who remained cognitively stable during follow-up. When we compared the CIND patients who subsequently converted to dementia with the stable CIND, we found that converters had smaller hippocampi. This is consistent with a previous report that showed baseline hippocampal volume was predictive of subsequent conversion to AD in older MCI patients.¹¹ We also found reduced neocortical GM volume in CIND-converters relative to stable CIND patients. This cross-sectional finding is similar to the results of a longitudinal imaging study that showed greater rates of whole-brain atrophy in subjects who declined cognitively than subjects who remained stable.³³ MTL NAA differences between CIND-converters and stable CIND patients did not reach significance; however, this may be due to sample size limitations. The fact that

MTL NAA concentrations in the two CIND groups differed by virtue of their comparison with the control group suggests an interesting potential source of clinical differentiation that should be examined more closely in a larger sample. Finally, it is noteworthy that receiver operator characteristic analysis revealed that sensitivity and specificity in separating CIND from control subjects increased when MTL NAA was used together with hippocampal volume compared to using hippocampal volume alone.

CIND patients had intermediate levels of parietal lobe GM NAA compared to AD patients and controls; however, between-group comparisons revealed no significant parietal GM NAA differences between CIND patients and controls or between CIND and AD patients. Our analysis of the Z-score data revealed that CIND patients had less negative parietal GM NAA Z-scores (left: -0.8, right: -0.6) than MTL NAA Z-scores (left: -1.1, right: -0.8). Together, these findings appear to be consistent with the known topographic progression of neurofibrillary pathology in AD beginning in the MTL and later spreading to upper levels of isocortical regions.³⁴

Considerable research has demonstrated the importance of MTL structures in the acquisition of new and the retrieval of previously learned information.³⁵ Moreover, some investigators have found correlations between MTL metabolic alterations and cognitive decline in AD patients.^{13,14} Consistent with these results, we found a positive correlation between reduced MTL NAA and impaired declarative memory performance in AD and CIND patients. This result underscores the biologic significance of reduced MTL NAA in older CIND patients who are at increased risk for developing dementia. A previous study found hippocampal volume to be predictive of declarative memory performance across the spectrum of normal aging to AD.¹² In this study, we also found a positive relationship between hippocampal volume and impaired declarative memory performance. However, unlike MTL NAA, hippocampal volume only correlated significantly with performance on the short-delay recall condition of the MAS word-list learning test. Although it is tempting, from a mechanistic point of view, to try to explain why we observed this particular pattern of results, the small number of subjects who had psychometric and imaging data prevented us from performing a more thorough analysis of the relationship between MTL NAA, hippocampal volume, and declarative memory performance. Thus, the nature of the relationship between volumetric and NAA measurements with different aspects of memory needs to be examined more closely in a larger subject sample.

This study had several limitations: 1) The sample sizes, especially in the stable CIND and CIND-converter groups, were small. 2) The make-up of the CIND group was heterogeneous. Consequently, the CIND-converters manifested different types of dementia at follow-up (i.e., AD, mixed, and vascular dementia). 3) There was incomplete follow-up information on the healthy controls. This is an important issue because the association between cognitive decline and age is variable despite the general increase in neurofibrillary pathology with age. We have follow-up information on 17 of the 24 healthy controls who participated in the study. Sixteen subjects remained cognitively stable after a mean follow-up interval of 3.5 ± 1.8 years. One subject's MMSE score declined from a baseline of 29 to 24 in 5 years; however, we do not have any additional clinical information on this individual. 4) The lack of psychometric data in a large number of controls and AD patients prevented us from conducting a more thorough examination of the relationship between MTL NAA, hippocampal volume, and declarative memory performance. 5) The use of a medium spin-echo acquisition time (TE = 135 msec) prohibited us from observing metabolites like mI, which have shorter T2 relaxation times. Given that mI elevation has been shown to be a reliable and specific marker of early dementia, particularly when combined with NAA,² future studies using single voxel MRS or other MRSI techniques that detect mI should be informative. 6) Some of the AD patients were taking cholinesterase inhibitors and anticholinergic drugs that could have altered brain metabolite levels, potentially enhancing metabolite differences between AD and controls and between AD

and CIND. 7) AD patients, especially those who were more impaired, were more likely to have moved during the MRI/MRSI scanning session than control subjects or CIND patients. This would have contributed to increased variability of the data in the AD group. These limitations notwithstanding, our results suggest that reduced MTL NAA, in addition to reduced hippocampal and neocortical GM volumes, may be an early indication of dementia-related pathology in elderly individuals at high risk to develop dementia.

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Figure.

Examples of N-acetylaspartate (NAA) images and representative proton spectra obtained from the medial temporal lobe (MTL) region, including the hippocampus and entorhinal cortex (location indicated by a circle in the MRI and NAA images) in a healthy control (HC), a cognitively impaired but nondemented (CIND) patient, and a patient with Alzheimer disease (AD). The indicated size for the voxel in the figure approximates the nominal spatial resolution of the MRSI. Also shown are the corresponding axial MR images for anatomic reference of the low-resolution NAA images. Note that MRI and NAA images have different fields of view. Reduction of NAA in the CIND and AD patients with respect to the healthy control subject becomes apparent when the NAA peak intensity in each spectrum is compared with creatine (Cr).

Table 1

Demographic and clinical information

	Control	AD	CIND		
			All	Converted to dementia	Remained stable
n	24	24	17	6	6
Male:female ratio	12:12	14:10	12:5	3:3	5:1
Age, y	76.0 ± 6.3	74.8 ± 6.9	75.4 ± 6.8	78.0 ± 6.3	74.7 ± 6.2
CDR	0.0 ± 0.0	1.0 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
MMSE	29.0 ± 0.8	17.4 ± 6.7*	26.6 ± 2.8	27.0 ± 2.2	27.2 ± 1.9
WMH	8.4 ± 7.7	12.0 ± 14.6	14.0 ± 17.0	23.9 ± 18.7	13.0 ± 11.1

Values are mean ± SD.

AD = Alzheimer disease; CIND = cognitively impaired but nondemented; CDR = Clinical Dementia Rating; MMSE = Mini-Mental State Examination; WMH = white matter hyperintensities (percent of intracranial volume).

* $p < 0.0001$ vs controls and vs CIND.

Table 2

Hippocampal, neocortical gray matter (GM), and total intracranial volume*

	Control	AD	CIND		
			All	Converted	Stable
L hippocampus	2.26 ± 0.24	1.57 ± 0.38	1.93 ± 0.40	1.75 ± 0.19	2.24 ± 0.27
R hippocampus	2.24 ± 0.26	1.74 ± 0.37	1.99 ± 0.30	1.85 ± 0.16	2.14 ± 0.26
Neocortical GM	509.24 ± 40.04	480.78 ± 42.25	482.62 ± 41.22	444.29 ± 30.35	497.75 ± 32.92
Total intracranial volume	1,325.60 ± 128.07	1,328.70 ± 156.49	1,381.89 ± 179.90	1,378.69 ± 280.23	1,365.50 ± 98.06

Values are mean ± SD.

AD = Alzheimer disease; CIND = cognitively impaired but nondemented.

* Volumes have been normalized to intracranial volume and scaled to the mean intracranial volume of all subjects (mean group intracranial volume/individual intracranial volume) to maintain volume in units of cm³.

NAA concentration in the MTL* in arbitrary units, relative to Cr concentration, and the proportion of gray matter to brain tissue (GM index) in the spectroscopy voxels

	Side	Control	AD	CIND		
				All	Converted	Stable
NAA in arbitrary units	L	2.38 ± 0.57	1.90 ± 0.45	1.87 ± 0.52	1.76 ± 0.35	2.08 ± 0.37
	R	2.43 ± 0.56	1.82 ± 0.52	1.96 ± 0.48	2.00 ± 0.58	2.09 ± 0.29
NAA/Cr ratio	L	1.71 ± 0.58	1.38 ± 0.42	1.39 ± 0.25	1.39 ± 0.30	1.46 ± 0.26
	R	1.64 ± 0.32	1.43 ± 0.52	1.39 ± 0.30	1.39 ± 0.26	1.41 ± 0.31
GM index	L	0.48 ± 0.14	0.46 ± 0.15	0.53 ± 0.13	0.60 ± 0.16	0.49 ± 0.12
	R	0.49 ± 0.09	0.51 ± 0.12	0.51 ± 0.10	0.54 ± 0.09	0.48 ± 0.09

Values are mean ± SD.

NAA = N-acetylaspartate; MTL = medial temporal lobe; GM index = [gray matter/(gray matter + white matter)]; AD = Alzheimer disease; CIND = cognitively impaired but nondemented.

* Includes the hippocampus and entorhinal cortex.

Table 4
concentration in the parietal and frontal lobe gray matter in arbitrary units

	Side	Control	AD	CIND		
				All	Converted	Stable
Parietal	L	2.09 ± 0.34	1.62 ± 0.56	1.80 ± 0.43	1.78 ± 0.58	1.61 ± 0.32
	R	2.13 ± 0.42	1.77 ± 0.44	1.88 ± 0.50	1.64 ± 0.40	1.99 ± 0.62
Frontal	L	2.15 ± 0.34	1.94 ± 0.43	1.89 ± 0.44	1.72 ± 0.55	1.95 ± 0.23
	R	2.11 ± 0.35	1.89 ± 0.44	1.93 ± 0.26	1.80 ± 0.27	1.99 ± 0.22

Values are mean ± SD.

NAA = N-acetylaspartate; AD = Alzheimer disease; CIND = cognitively impaired but nondemented.

Table 5

Classification of CIND patients and healthy controls

Measures	Sensitivity	Specificity	Overall	ROC	p Value *
Hippocampal volume	0.38	0.87	0.77	0.78	0.02
+ MTL NAA	0.40	0.87	0.76	0.84	0.03
+ Neocortical gray matter	0.38	0.87	0.77	0.79	0.15

Values are mean ± SD. Sensitivity, specificity, and overall correct classification were calculated using logistic regression analysis in 24 controls and 17 CIND patients.

CIND = cognitively impaired but nondemented; ROC = receiver operator characteristic area under the curve; MTL NAA = medial temporal lobe *N*-acetylaspartate.

* Analysis by logistic regression.