Hippocampal and entorhinal atrophy in mild cognitive impairment

Prediction of Alzheimer disease

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Abstract—*Objective:* To evaluate the utility of MRI hippocampal and entorhinal cortex atrophy in predicting conversion from mild cognitive impairment (MCI) to Alzheimer disease (AD). *Methods:* Baseline brain MRI was performed in 139 patients with MCI, broadly defined, and 63 healthy controls followed for an average of 5 years (range 1 to 9 years). *Results:* Hippocampal and entorhinal cortex volumes were each largest in controls, intermediate in MCI nonconverters, and smallest in MCI converters to AD (37 of 139 patients converted to AD). In separate Cox proportional hazards models, covarying for intracranial volume, smaller hippocampal volume (risk ratio [RR] 3.62, 95% CI 1.93 to 6.80, $p < 0.0001$), and entorhinal cortex volume (RR 2.43, 95% CI 1.56 to 3.79, $p < 0.0001$) each predicted time to conversion to AD. Similar results were obtained for hippocampal and entorhinal cortex volume in patients with MCI with Mini-Mental State Examination (MMSE) scores ≥ 27 out of 30 (21% converted to AD) and in the subset of patients with amnestic MCI (35% converted to AD). In the total patient sample, when both hippocampal and entorhinal volume were entered into an age-stratified Cox model with sex, MMSE, education, and intracranial volume, smaller hippocampal volume (RR 2.21, 95% CI 1.14 to 4.29, $p < 0.02$) and entorhinal cortex volume (RR 2.48, 95% CI 1.54 to 3.97, $p < 0.0002$) predicted time to conversion to AD. Similar results were obtained in a Cox model that also included Selective Reminding Test (SRT) delayed recall and Wechsler Adult Intelligence Scale–Revised (WAIS-R) Digit Symbol as predictors. Based on logistic regression models in the 3-year follow-up sample, for a fixed specificity of 80%, the sensitivities for MCI conversion to AD were as follows: age 43.3%, MMSE 43.3%, age + MMSE 63.7%, age + MMSE + SRT delayed recall + WAIS-R Digit Symbol 80.6% (79.6% correctly classified), hippocampus + entorhinal cortex 66.7%, age + MMSE + hippocampus + entorhinal cortex 76.7% (85% correctly classified), age + MMSE + SRT delayed recall + WAIS-R Digit Symbol + hippocampus + entorhinal cortex 83.3% (86.8% correctly classified). *Conclusions:* Smaller hippocampal and entorhinal cortex volumes each contribute to the prediction of conversion to Alzheimer disease. Age and cognitive variables also contribute to prediction, and the added value of hippocampal and entorhinal cortex volumes is small. Nonetheless, combining these MRI volumes with age and cognitive measures leads to high levels of predictive accuracy that may have potential clinical application.

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The medial temporal lobe, which includes the hippocampus and parahippocampal gyrus (the latter includes the entorhinal cortex), atrophies early in Alzheimer disease (AD).[1] High-resolution T1-weighted MRI allows precise assessment of these structures. In AD, hippocampal volume is smaller than in controls and is associated with greater dementia severity.[2-4] Similarly, entorhinal cortex volume is smaller in AD vs controls.[5,6]

In patients with mild cognitive impairment (MCI), a condition that is often transitional to AD,[7,8] hippocampal and entorhinal cortex volumes lie between the values measured in controls and AD.[4,9,10] In an epidemiologic sample, entorhinal atrophy was greater than hippocampal atrophy in MCI, but the two measures did not differ in AD, suggesting that the entorhinal cortex atrophies before the hippocampus in incipient AD.[10] Autopsy studies in early AD show neurofibrillary tangles in the entorhinal cortex before hippocampal involvement.[11]

The predictive utility and relative merit of hippocampal[12] and entorhinal cortex atrophy[13] for MCI conversion to AD are not established, partly because of sample size limitations.[6,13] Another clinically important issue is the predictive utility of these volumetric measures in relation to age, sex, education,

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and cognitive measures.[12-14] To address these issues, we evaluated baseline MRI hippocampal and entorhinal cortex (and parahippocampal gyrus) volumes in a single-site, long-term follow-up study of a large cohort of cognitively impaired outpatients without dementia and an age- and sex-matched control group.

Methods. *Subjects.* Patients presented with memory complaints to the Memory Disorders Center at New York State Psychiatric Institute and Columbia-Presbyterian Medical Center. The majority (52%) were physician referred, 25% were self-referred, and 23% were referred by family or friends or other sources. The inclusion/exclusion criteria aimed at enrolling a broad sample of cognitively impaired outpatients who presented with memory complaints and were found to have cognitive impairment without dementia based on comprehensive evaluation, but without a specific diagnosable cause for the cognitive impairment. Inclusion criteria were age $\geq$ 40 years, cognitive impairment $\geq$ 6 months and $\leq$ 10 years, and a minimum modified Mini-Mental State Examination (MMSE) score $\geq$ 40 out of 57 (Folstein MMSE $\geq$ 22 out of 30).[15] Neuropsychological inclusion guidelines were Folstein MMSE recall $\leq$ 2 out of 3 objects at 5 minutes, or a Selective Reminding Test (SRT) delayed recall score $>$ 1 SD below norms, or a Wechsler Adult Intelligence Scale–Revised (WAIS-R) performance IQ score $\geq$ 10 points below the WAIS-R verbal IQ score. Patients without these neuropsychological deficits were eligible if they met all three of the following criteria: subjective complaint of memory decline, informant's confirmation of decline, and total score $\geq$ 1 on the first eight items of the modified Blessed Functional Activity Scale.[15]

Exclusion criteria were a diagnosis of dementia, schizophrenia, current major affective disorder, alcohol or substance dependence, history of stroke, cortical stroke or infarct $\geq$ 2 cm in diameter based on MRI, cognitive impairment entirely caused by medications, or other major neurologic illness, e.g., Parkinson disease.

Healthy controls were recruited primarily by advertisement. Inclusion criteria were the absence of memory complaints, score $\geq$ 27 out of 30 on the MMSE with recall $\geq$ 2 out of 3 objects at 5 minutes, and neuropsychological test scores not more than 1 SD below age-adjusted norms. Medical, neurologic, and psychiatric exclusion criteria were the same as for patients. Healthy controls were group-matched to the patients on age and sex. All subjects signed informed consent in this institutional review board–approved protocol.

Procedures. The study neurologist/psychiatrist obtained history and conducted a general physical, neurologic, and psychiatric examination. Laboratory tests included complete blood count, serum electrolytes, liver and renal function tests, thyroid function tests, Venereal Disease Research Laboratory/rapid plasma reagin tests, and serum B_{12} and folate levels. Apolipoprotein E genotype was assessed, and patients were characterized as ε4 carriers (ε3/ε4 or ε2/ε4 or ε4/4) or ε4 noncarriers.

Patients and controls received annual neuropsychological testing, reviewed by an experienced neuropsychologist (Y.S.). Patients completed the MMSE and SRT at intervening 6-month intervals. At baseline, two expert clinical raters (D.P.D. and Y.S.) used neuropsychological performance, clinical and laboratory test results, and the radiologist's MRI clinical report to reach a consensus diagnosis, while remaining blind to apolipoprotein E genotype and MRI volumetric data. At each follow-up, a similar consensus diagnosis was made by the same raters who remained blind to data from previous visits. The diagnosis of dementia was based on *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition criteria, and the diagnosis of possible or probable AD on National Institute of Neurological and Communicative Diseases and Stroke–Alzheimer's Disease and Related Disorders Association criteria.[16] The endpoint of AD required a diagnosis of possible or probable AD at two consecutive annual assessments. The consensus diagnosis was the primary outcome variable.

These inclusion/exclusion criteria and diagnostic procedures remained constant throughout this prospective, longitudinal study. Four years after this study began, the Petersen MCI criteria were defined.[7] For the purpose of secondary analysis, based on Petersen guidelines,[7] MCI subtype was derived from the following neuropsychological test data already collected at baseline: 1) memory (verbal and nonverbal): SRT total and delayed recall, Wechsler Memory Scale visual reproduction immediate and delayed recall; 2) executive functions: WAIS-R similarities, WAIS-R Digit Symbol, letter fluency; 3) language: Boston Naming, Animal Naming, Boston Diagnostic Aphasia Evaluation Comprehension, Repetition; 4) visuospatial: Rosen Drawing Test, WAIS-R object assembly, WAIS-R block design. As described elsewhere,[17] age-, education-, and sex-based regression norms for these tests were calculated for 83 healthy elderly controls (included 63 controls from this study). Using the criterion of $>$ 1.5 SD below these norms on any test within each domain, the 139 patients with complete MRI data were classified as MCI–amnestic single domain (n = 21), MCI amnestic plus other cognitive domain deficits (n = 80), MCI–executive (n = 2), MCI–language (n = 12), MCI–visuospatial (n = 2), MCI–multiple domain deficits without memory (n = 5), and non-MCI (cognitive deficits not meeting 1.5 SD threshold, n = 17).

MRI scan acquisition. At baseline, each subject had a brain MRI acquired on the same GE 1.5-T Signa 5X unit. The following sequences were obtained: 1) T1-weighted sagittal scout images using spin echo sequence with repetition time (TR): 550, echo time (TE): 10, 5-mm slice thickness, 2.5-mm gap, matrix: 256 $\times$ 192 $\times$ 1 NEX, and 24-cm field of view (FOV); 2) T1-weighted axial images parallel to the temporal horns using a spin echo sequence with TR: 550, TE: 11, 5-mm slice thickness without gap, matrix: 256 $\times$ 192 $\times$ 1 NEX, and 24-cm FOV; 3) proton density and T2-weighted fast spin echo coronal images, perpendicular to the temporal horns, acquired with a dual echo sequence with an 8-echo train, TR: 4,000, TE: 17 and 102, 5-mm slice thickness without gap, matrix: 256 $\times$ 192 $\times$ 1 NEX, and 20 cm FOV; 4) three-dimensional coronal volume spoiled gradient recalled echo (SPGR) sequence, perpendicular to the temporal horns, with TR: 34, TE: 13, flip angle 45 degrees, 2-mm-thick contiguous slices, matrix 256 $\times$ 256 $\times$ 1 NEX, and a rectangular FOV of 24 $\times$ 18 cm.

MRI processing. A single rater (G.P.) evaluated all scans on a Sun UltraSPARC workstation blind to all clinical information, using a dedicated software package (MIDAS) for image segmentation and coregistration.[18] To evaluate hippocampus, parahippocampal gyrus, and entorhinal cortex volumes, images from the coronal SPGR sequence were realigned to a standard orientation and reformatted using sinc interpolation to a 2-mm slice thickness in the coronal plane. The standard alignment was based on the interhemispheric fissure, the lens of both eyes, and in the sagittal plane the line connecting the anterior and posterior commissures. Some MRI scans did not have whole brain coverage with the coronal SPGR sequence. Therefore, to estimate intracranial volume, the T1 axial 5-mm-thick images were reformatted using sinc interpolation to sagittal images of 5-mm slice thickness. Supratentorial intracranial volumes were defined along the inner table of the skull, and in midsagittal slices, the inferior boundaries were the sphenoid bone and suprasellar region excluding the pituitary gland and optic nerve or chiasm.

For the hippocampal volume assessment method, postmortem anatomic validation has been reported.[19] The lateral border of the hippocampus was the medial wall of the temporal horn; the anterior boundary was the amygdala with the transition cortex between the amygdala cortical nucleus and hippocampus excluded by making a perpendicular section at the level of the semilunar gyrus. Anteriorly, the superior border was defined by the temporal horn and fimbria, the medial border by the ambient cistern, and the inferior border by the uncal sulcus and parahippocampal gyrus. More posteriorly, the hippocampal body's medial boundary was the transverse fissure, and its inferior boundary was the parahippocampal gyrus (medial part of the subiculum if the uncal sulcus was not visible). Because the interface between the lateral portion of the subiculum and the hippocampus (Ammon's horn) cannot be distinguished, this portion was included in the hippocampus and not the parahippocampal gyrus (figures 1 and 2).

The parahippocampal gyrus volume included the medial portion of the subiculum, the entorhinal cortex, the transentorhinal cortex, and the parahippocampal neocortex and white matter. The medial border was the ambient cistern, and the inferior border was the tentorium cerebelli (figure 2). In anterior sections, the superior border of the parahippocampal gyrus was the hippocampus and the uncal sulcus. In more posterior sections, the superior boundary was the hippocampus laterally and the transverse fis-

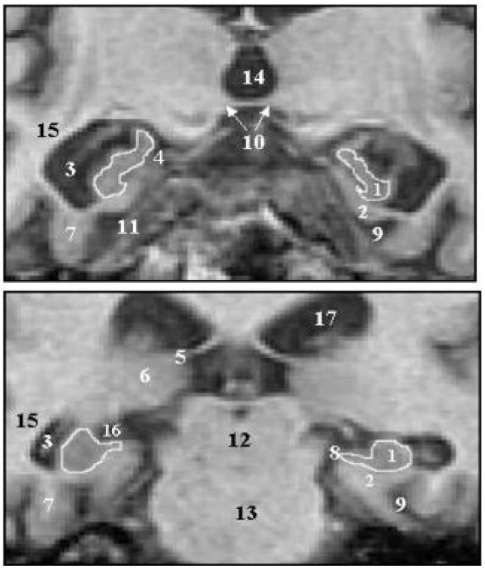

Figure 1. Magnified coronal MRI images of anterior (upper image) and posterior (lower image) hippocampal slices of a 67-year-old male patient with MCI who subsequently converted to Alzheimer disease. The anterior slice is at the level of the hippocampal–amygdalar transitional area, and the posterior slice is at the level where the pulvinar surrounds the crus of the fornix. 1 = Hippocampus; 2 = parahippocampal gyrus; 3 = temporal horn of lateral ventricle; 4 = uncus; 5 = crus of fornix; 6 = pulvinar; 7 = fusiform gyrus; 8 = ambient cistern; 9 = collateral sulcus; 10 = mammillary body; 11 = tentorium cerebelli; 12 = cerebral aqueduct; 13 = pons; 14 = third ventricle; 15 = temporal stem; 16 = transverse fissure; 17 = lateral ventricle.

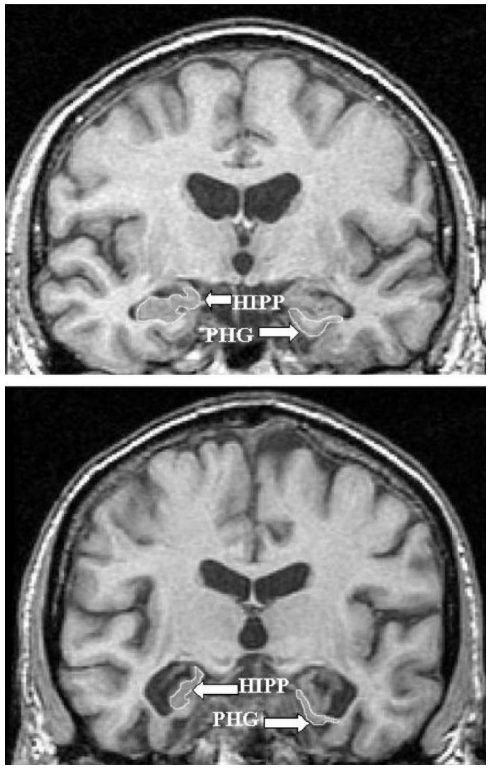

Figure 2. Baseline coronal MRI slices, 2 mm posterior to the hippocampal–amygdalar junction, showing the tracings for anterior hippocampus and parahippocampal gyrus. Upper image: 70-year-old healthy control male subject. Lower image: 67-year-old male patient with mild cognitive impairment who subsequently converted to Alzheimer disease. HIPP = hippocampus; PHG = parahippocampal gyrus.

sure. The medial part of the subiculum (presubiculum and parasubiculum) was included in the parahippocampal gyrus volume. The anterior and posterior boundaries of the parahippocampal gyrus corresponded to the same level (slice) as the anterior and posterior boundaries of the hippocampus.[9] This approach standardized the procedures across all subjects but may have led to exclusion of the anterior-most and posterior-most portions of both structures.

The entorhinal cortex was assessed by estimating the volume across three slices according to the method validated by Killiany et al.[6] First, the mammillary bodies were identified. The first (most rostral) image displaying the fornix white matter tracts was the center image, and the adjacent anterior and posterior slices were then identified. The outline began at the angle formed by the junction of the rhinal sulcus and the brain surface and then transected the angle formed by the rhinal sulcus and the inferomedial brain surface. The line cut across the gray matter and then followed the lower edge of the white matter to the inferior hippocampal surface and down along the brain surface to the initial outline point (figure 3). The outlines used the same landmarks on all three slices; the entorhinal cortex volume assessment averaged 15 minutes after identifying the correct image containing the mammillary bodies.[6] This method restricts the entorhinal cortex assessment to the region where it is relatively large and the boundaries are clear, enhancing reliability.

Statistics. Analysis of variance, analysis of covariance, and χ^2 test were used to compare the demographic, clinical, and MRI variables of patients (converters, nonconverters to AD) and controls. The *t* test was used for two-group comparisons of the MRI variables. Pearson correlation coefficients were used to describe the relationships among the MRI and neuropsychological variables.

The time to AD was defined from the initial visit to the first follow-up time-point at which AD was diagnosed. Primary analyses were conducted in the entire patient sample. Age-stratified Cox proportional hazards models were used to examine the separate effects of hippocampal, parahippocampal gyrus, or entorhinal cortex volume (left + right), covarying for intracranial volume, on the time to conversion to AD with and without further controlling for sex, education, and baseline MMSE, as well as SRT delayed recall and WAIS-R Digit Symbol scores, which were the strongest among the hypothesized neuropsychological predictors of time to conversion to AD, as reported elsewhere.[20] Secondary Cox analyses were conducted in two subsamples of patients from within the broad patient sample: the subsample with baseline MMSE $\geq$ 27 out of 30 (n = 99) and the subsample with amnestic MCI (n = 101), which is the group at high risk of conversion to AD.

In all patients who completed 3-year follow-up or converted to AD before 3-year follow-up, logistic regression analyses were conducted and sensitivities and specificities were calculated for receiver operating characteristic curves. For a fixed specificity of 80%, sensitivity was calculated. Percent correctly classified was computed based on the threshold of 0.5 on the predicted risk derived from the logistic model.

Results. *Demographic and clinical features.* Within 6 months of presentation, two patients with MCI were diagnosed with other neurologic disorders (corticobasal degeneration, and ALS presenting with frontal lobe deficits) and were excluded. MCI converters, MCI nonconverters, and controls did not differ in sex distribution, years of education, and apolipoprotein E ε4 carrier status (table 1). MCI converters were older and scored lower on baseline MMSE than MCI nonconverters or controls (table 1). Two control subjects cognitively declined and met criteria for amnestic MCI during follow-up, and none converted to AD.

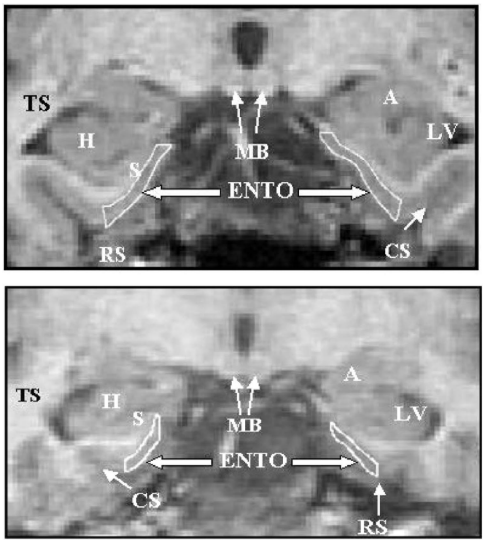

Figure 3. Illustration of entorhinal cortex volume assessed by the Killiany method. Three slices are evaluated by this method; only the central slice at the level of the mammillary bodies is shown. In each slice, the boundaries of the entorhinal cortex are enhanced for visual clarity. Upper image: 71-year-old female, healthy control subject. Lower image: 66-year-old female, patient with MCI who converted to Alzheimer disease. ENTO = entorhinal cortex; MB = mammillary bodies; RS = rhinal sulcus; A = amygdala; H = hippocampus; S = subiculum; CS = collateral sulcus; LV = lateral ventricle.

Interrater and intrarater reliability. The single MRI rater trained with expert raters (M.D.L., S.D.S., and S.S.) and showed high interrater reliability on 10 scans (sum of left and right volumes): hippocampal volume intraclass correlation coefficient (ICC) = 0.90, parahippocampal gyrus volume ICC = 0.96, and entorhinal cortex volume ICC = 0.92. The rater blindly re-rated 10 scans that she had rated more than a year earlier and showed high intrarater reliability: hippocampal volume ICC = 0.98, parahippocampal gyrus volume ICC = 0.97, and entorhinal cortex volume ICC = 0.99.

Baseline hippocampal and parahippocampal gyrus and entorhinal cortex volumes. MRI was conducted in 140 patients and 63 controls, but one patient and one control were excluded because of extensive head motion. Hippocampal and entorhinal volumes were lower in patients with amnestic MCI compared with the rest of the patient sample and controls. In analyses of covariance restricted to the 3-year follow-up sample to avoid converter/nonconverter bias, all three medial temporal lobe volumes (covarying for intracranial volume) increased from MCI converters to MCI nonconverters to controls (table 1).

In patients, neither hippocampal nor entorhinal cortex volumes were related to sex, education, or apolipoprotein E ε4 carrier status. In patients, hippocampal volume decreased with age ($r = -0.33$, $p < 0.001$) and increased with baseline MMSE ($r = 0.28$, $p < 0.001$), SRT delayed recall ($r = 0.31$, $p < 0.001$), and WAIS-R Digit Symbol scores ($r = 0.19$, $p < 0.03$). Entorhinal cortex volume decreased with age ($r = -0.25$, $p < 0.005$) and increased with baseline WAIS-R Digit Symbol scores ($r = 0.18$, $p < 0.04$), but not with MMSE ($r = 0.08$, $p = 0.38$) or SRT delayed recall ($r = 0.12$, $p = 0.16$).

Follow-up. Follow-up duration averaged nearly 5 years in controls (table 1). In patients, converters had shorter follow-up because they exited the study after two consecutive annual AD diagnoses (table 1). Of the 15

Table 1 *Baseline features of patients with MCI (converters and nonconverters to AD) and healthy control subjects*

	Patients with MCI		Healthy controls	
Feature	37 Converters	102 Nonconverters	63 Controls	$p^* <$
Sex, % female	56.8	55.6	54.8	0.99
Baseline age, mean ± SD, years	72.2 ± 7.1	64.8 ± 10	65.6 ± 9.4	0.001
Education, mean ± SD, years	14.1 ± 4.5	15.6 ± 4.0	16.8 ± 2.6	0.16
Baseline MMSE, mean ± SD	26.2 ± 2.2	28.0 ± 2.0	29.4 ± 0.8	0.001
Apolipoprotein E ε4 carrier, %	32	20	21	0.30
Follow-up duration, years†	3.43 ± 1.59	4.52 ± 2.22	4.95 ± 2.41	0.01
Hippocampal volume (R + L)‡	3.49 ± 0.84	3.94 ± 0.72	4.34 ± 0.57	0.0001
Parahippocampal gyrus volume (R + L)‡	7.0 ± 1.20	7.57 ± 1.24	7.79 ± 1.24	0.0001
Entorhinal cortex volume (R + L)‡	0.387 ± 0.095	0.466 ± 0.087	0.548 ± 0.096	0.0001
Supratentorial intracranial volume, cc	1,281 ± 118	1,310 ± 131	1,318 ± 134	0.39

All values are means ± standard deviations, or percentages.

* Significance level for χ^2 or analysis of variance (analysis of covariance for hippocampal, parahippocampal gyrus and entorhinal cortex volumes, covarying for intracranial volume).

† Converters to Alzheimer disease (AD) exited the study after two consecutive annual AD diagnoses, thereby reducing the follow-up duration in this group.

‡ Hippocampal, parahippocampal gyrus, and entorhinal cortex volumes are in cubic centimeters.

In analyses of covariance, hippocampal and parahippocampal gyrus and entorhinal cortex volumes were analyzed for converters/nonconverters/controls restricted to the 3-year follow-up sample, covarying for intracranial volume.

MCI = mild cognitive impairment; MMSE = 30-item Folstein Mini-Mental State Examination.

Table 2. *Hazards ratios (95% CI) derived from age-stratified (quartiles: 42–59, 60–67, 68–74, 75–85 years) Cox proportional hazards models with the main predictors of the specific MRI variables, covarying for ICV with and without further adjusting for sex, education, MMSE scores, SRT delayed recall, and WAIS-R Digit Symbol in specific combinations as listed below*

	All patients (37/139 converted to AD)		Amnestic MCI (35/101 converted to AD)	
	Hazards ratio (95% CI)	*p* Value	Hazards ratio (95% CI)	*p* Value
MRI brain volume as predictor, covarying for ICV				
Hippocampus*	3.62 (1.93–6.80)	<0.0001	2.84 (1.47–5.49)	0.0018
Entorhinal cortex†	2.43 (1.56–3.79)	<0.0001	2.31 (1.50–3.56)	0.0002
Hippocampus‡	2.98 (1.55–5.75)	0.0011	2.29 (1.16–4.52)	0.0173
Entorhinal cortex‡	2.14 (1.38–3.31)	0.0007	2.12 (1.38–3.26)	0.0006
Adjusted for ICV, sex, education, MMSE				
Hippocampus*	2.89 (1.52–5.51)	0.0012	2.31 (1.15–4.67)	0.0192
Entorhinal cortex†	2.79 (1.75–4.47)	<0.0001	3.06 (1.86–5.00)	<0.0001
Hippocampus‡	2.21 (1.14–4.29)	0.0196	1.47 (0.68–3.16)	0.33
Entorhinal cortex‡	2.48 (1.54–3.97)	0.0002	2.83 (1.70–4.72)	<0.0001
Adjusted for ICV, sex, education, SRT delayed recall, WAIS-R Digit Symbol				
Hippocampus*	2.63 (1.32–5.26)	0.0062	2.42 (1.15–5.11)	0.0205
Entorhinal cortex†	2.92 (1.68–5.08)	0.0002	2.73 (1.57–4.74)	0.0004
Hippocampus‡	2.31 (1.12–4.77)	0.0232	2.18 (0.97–4.90)	0.0591
Entorhinal cortex‡	2.69 (1.57–4.60)	0.0003	2.58 (1.51–4.43)	0.0006
Adjusted for ICV, sex, education, MMSE, SRT delayed recall, WAIS-R Digit Symbol				
Hippocampus*	2.51 (1.24–5.06)	0.0104	2.24 (1.04–4.83)	0.0387
Entorhinal cortex†	3.14 (1.82–5.42)	<0.0001	3.29 (1.86–5.83)	<0.0001
Hippocampus‡	1.97 (0.95–4.08)	0.0679	1.57 (0.67–3.67)	0.2993
Entorhinal cortex‡	2.88 (1.68, 4.93)	0.0001	3.06 (1.72–5.45)	0.0001

* Hippocampal volume: hazards ratios are per cc.
† Entorhinal cortex volume: hazard ratios are per 0.1 cc.
‡ Hippocampus and entorhinal cortex included as predictors within the same model.

ICV = intracranial volume; AD = Alzheimer disease; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; SRT = Selective Reminding Test; WAIS-R = Wechsler Adult Intelligence Scale–Revised.

deaths during follow-up, there were 4 autopsies, all of which confirmed the clinical diagnoses (3 AD, 1 healthy control).

In the total patient sample (n = 139), in separate age-stratified Cox proportional hazards models with intracranial volume as covariate, smaller volumes of the hippocampus (risk ratio [RR] per 1 mL volume reduction = 3.62, 95% CI 1.93 to 6.80, $p < 0.0001$) and entorhinal cortex (RR per 0.1 mL volume reduction = 2.43, 95% CI 1.56 to 3.79, $p < 0.0001$) were each associated with the hazard of conversion to AD (table 2). After adjusting for sex, education, baseline MMSE, and intracranial volume, in separate Cox analyses, smaller hippocampal ($p < 0.0012$) and entorhinal cortex volume ($p < 0.0001$) each remained strong predictors (table 2). When both hippocampal and entorhinal cortex volumes were entered into the same model covarying for intracranial volume, hippocampal (RR 2.98, 95% CI 1.55 to 5.75, $p < 0.0012$) and entorhinal cortex (RR 2.14, 95% CI 1.38 to 3.31, $p < 0.0007$) volumes were strong predictors. Similar results were obtained when both hippocampal and entorhinal cortex volume were included in an age-stratified Cox model after adjusting for intracranial volume, sex, education, and MMSE, but with reduced significance levels for hippocampal volume (table 2). In similar Cox models that also included SRT delayed recall and WAIS-R Digit Symbol as predictors, entorhinal cortex volume remained highly significant, with less significance for hippocampal volume (table 2).

The Kaplan–Meier curves (figures 4 and 5) indirectly suggested that hippocampal atrophy showed progressive increase in risk for conversion to AD across the range of atrophy, whereas entorhinal cortex showed marked increase in risk mainly in those patients with volumes in the lowest tertile.

In secondary Cox analyses in the 99 patients with MCI (21 converters) with baseline MMSE ≥ 27 out of 30, smaller hippocampal (RR 3.44, 95% CI 1.61 to 7.35, $p < 0.0015$) and entorhinal cortex (RR 2.77, 95% CI 1.59 to 4.83, $p < 0.0003$) volumes were each associated with the hazard of conversion to AD.

In secondary analyses in the amnestic MCI subsample (n = 101), the results for hippocampus and entorhinal cortex volume were similar but with less significance (ta-

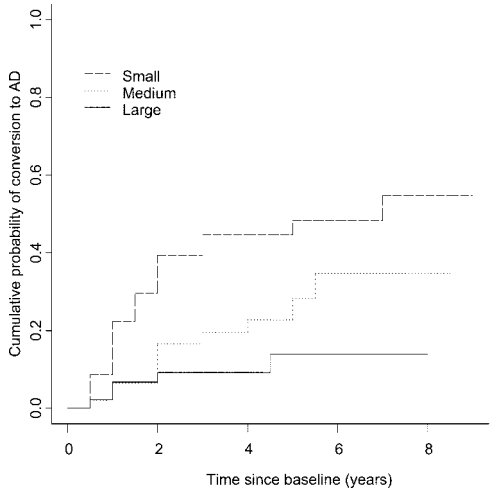

Figure 4. Kaplan–Meier curves for baseline hippocampal volume (right + left) in 139 patients with mild cognitive impairment for the cumulative probability of conversion to Alzheimer disease (AD). Hippocampal volume was trichotomized to generate the three curves: small volume 2.266 mL to 4.010 mL, n = 46; medium volume 4.010 mL to 4.470 mL, n = 47; large volume 4.470 mL to 5.95 mL, n = 46.

ble 2). In patients with amnestic MCI, entorhinal cortex volume (RR 2.83, 95% CI 1.70 to 4.72, $p < 0.0001$) but not hippocampal volume (RR 1.47, 95% CI 0.68 to 3.16, $p = 0.33$) remained a significant predictor when both entorhinal cortex and hippocampal volume were entered into an age-stratified Cox model after adjusting for intracranial volume, sex, education, and baseline MMSE (table 2).

For clinical relevance, further Cox analyses were conducted to estimate the relative risk of conversion in patients with hippocampal or entorhinal cortex volumes dichotomized at 10% or 20% below the corresponding mean volumes for control subjects (4.34 mL for hippocampus and 0.548 mL for entorhinal cortex). Covarying for intracranial volume in each Cox model, for hippocampal volume 10% below the control mean, the RR was 3.42 ($p < 0.001$, 95% CI 1.65 to 7.07, $p < 0.001$); for hippocampal volume 20% below the control mean, the RR was 10.1 ($p < 0.0001$, 95% CI 4.02 to 25.2); for entorhinal cortex volume 10% below the control mean, the RR was 2.50 ($p < 0.05$, 95% CI 1.03 to 6.06); and for entorhinal cortex volume 20% below the control mean, the RR was 4.17 ($p < 0.0005$, 95% CI 1.96 to 8.85).

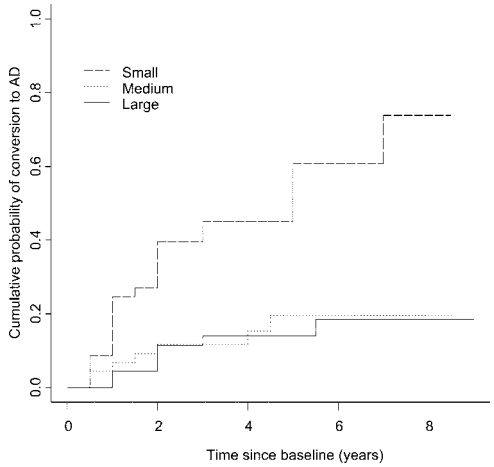

Figure 5. Kaplan–Meier curves for baseline entorhinal volume (right + left) in 138 patients with mild cognitive impairment for the cumulative probability of conversion to Alzheimer disease (AD). Entorhinal volume was trichotomized to generate the three curves: small volume 0.183 mL to 0.396 mL, n = 46; medium volume 0.396 mL to 0.480 mL, n = 46; large volume 0.480 mL to 0.705 mL, n = 46.

In Cox analyses, parahippocampal gyrus volume predicted conversion to AD with intracranial volume as covariate (RR per 1 mL volume reduction 1.80, 95% CI 1.21 to 2.68, $p < 0.004$). However, when both hippocampal and parahippocampal gyrus volumes were entered into the same model covarying for intracranial volume, both with and without further controlling for sex, education, and MMSE, hippocampal volume remained significant, but parahippocampal gyrus volume was not significant. Parahippocampal gyrus volume was not a significant predictor in any of the analyses that also included SRT delayed recall and WAIS-R Digit Symbol as predictors, and was not significant in analyses in the amnestic MCI subsample that included demographic and cognitive covariates.

Diagnostic sensitivity, specificity, and accuracy. Logistic regression analyses were conducted in patients who completed at least 3 years of follow-up or converted to AD before 3 years of follow-up (31 converters out of 114 patients). In these analyses (table 3), for a fixed specificity of 80%, the sensitivities for MCI conversion to AD were as follows: age 43.3%, MMSE 43.3%, age + MMSE 63.7%, age + MMSE + SRT delayed recall + WAIS-R Digit Symbol 80.6% (79.6% correctly classified), hippocampus + entorhinal cortex 66.7%, age + MMSE + hippocampus + entorhinal cortex 76.7% (85% correctly classified), age + MMSE + SRT delayed recall + WAIS-R Digit Symbol + hippocampus + entorhinal cortex 83.3% (86.8% correctly classified).

Intracranial volume did not predict conversion by itself ($p = 0.08$), nor after adjusting for age ($p = 0.11$) in the logistic regression model, nor in the age-stratified Cox proportional hazards model ($p = 0.44$).

Discussion. This sample is larger than that in other prospective longitudinal studies of MRI in MCI outpatients and confirms most of the findings reported in smaller samples.[6,12-14] Smaller hippocampal and entorhinal cortex volumes each were highly significant predictors of time to conversion to AD. We found that 1) hippocampal and entorhinal volume contributed significantly to prediction when both measures were evaluated in the same analysis; 2) hippocampal and entorhinal volume contributed to prediction over and above the effects of age and a measure of global cognition (MMSE), as well as episodic memory (SRT delayed recall) and attention/psychomotor/executive function (WAIS-R Digit Symbol); and 3) entorhinal cortex volume remained highly significant even after controlling for age and the cognitive measures, unlike hippocampal volume that became less significant in prediction in similar analyses partly because it correlated strongly with these cognitive measures.[6,21]

In logistic regression analyses in the 3-year

Table 3 *Predictive accuracy for classification of conversion to AD by 3 years of follow-up (31 of 114 patients converted to AD), using a threshold of 0.5 on predicted risk derived from the logistic regression models*

Predictor variable	*p* Value for effect of the predictor	Area under the curve	Sensitivity % for fixed specificity = 80%	Correct classification %
Age	0.001	0.703	43.3	71.9
MMSE	0.0003	0.770	43.9	74.3
SRT delayed recall	0.0001	0.814	67.3	78.1
WAIS-R Digit Symbol	0.0002	0.755	61.3	73.7
Age	0.0037	0.807	63.7	73.7
MMSE	0.0009			
Age	0.1753	0.876	80.6	79.6
MMSE	0.0467			
SRT delayed recall	0.0009			
WAIS-R Digit Symbol	0.0602			
HIP	0.0001	0.770	61.3	78.9
ENT	0.0002	0.762	63.3	79.6
HIP	0.001	0.812	66.7	86.7
ENT	0.002			
HIP	0.011	0.854	76.7	85.0
ENT	0.003			
Age	0.360			
MMSE	0.018			
HIP	0.0132	0.911	83.3	87.7
ENT	0.0114			
Age	0.8120			
SRT delayed recall	0.0161			
WAIS-R Digit Symbol	0.0192			
HIP	0.0343	0.912	83.3	86.8
ENT	0.0123			
Age	0.8924			
MMSE	0.3021			
SRT delayed recall	0.0266			
WAIS-R Digit Symbol	0.0300			

Area under the curve was derived from receiver operating characteristic analyses.

AD = Alzheimer disease; MMSE = Mini-Mental State Examination; SRT = Selective Reminding Test; WAIS-R = Wechsler Adult Intelligence Scale–Revised; HIP = hippocampus; ENT = entorhinal cortex.

follow-up sample, entorhinal cortex and hippocampal volume each showed moderately strong predictive accuracy. The combined effects of hippocampal and entorhinal cortex volumes further improved predictive accuracy. This indicates that each MRI measure independently contributes to prediction, but leaves open the question of whether the entorhinal cortex atrophies before the hippocampus in incipient AD.[1,10,13] Age and cognitive variables also contributed strongly to prediction, and the relatively small added value for prediction from the MRI volumes needs to be weighed against the costs and expertise required

to obtain them. Nonetheless, combining the hippocampal and entorhinal cortex volumes with age and cognitive measures did lead to the highest levels of predictive accuracy for the various combinations of variables that were evaluated (table 3).

The main findings held even in patients with MMSE $\geq$ 27 out of 30, which is the group with minimal global cognitive deficits that often presents diagnostic and prognostic difficulties for the clinician. This suggests potential utility for hippocampal and entorhinal atrophy in predicting conversion to AD even in patients with minimal to mild global cognitive deficits. Clinical and MRI radiologic readings were used to exclude patients with stroke or cortical or large lacunar infarctions, and it is unlikely that the presence of cerebrovascular disease played a major role in conversion to AD. Memory impairment, but not the degree of cerebrovascular disease, has been shown to predict MCI conversion to AD.[22]

In the total MCI sample, for hippocampal or entorhinal cortex volume reduction of 10% or 20% compared with the control mean volumes, the RR ranged from 2.5 to 10, indicating considerably increased risk for even mild degrees of atrophy in these key medial temporal regions. Hippocampal and entorhinal cortex volume loss ranges from 30% to 50% in studies of AD compared with healthy controls,[3,5,6,10] but it is less severe in MCI, e.g., entorhinal cortex volume loss 16% and hippocampal volume loss 8% in an epidemiologic study.[10] In our 3-year follow-up sample, mean hippocampal volume in converters was 11% lower than in nonconverters and 14% lower than in controls. Mean entorhinal cortex volume in converters was 17% lower than in nonconverters and 29% lower than in controls (table 1). Another study showed entorhinal cortex volume loss in MCI converters similar to mild AD patients.[6] This magnitude of volume loss has been validated by studies showing that hippocampal atrophy during life is corroborated by increased hippocampal neurofibrillary tangle burden after death.[23,24,25]

In MCI converters to AD, parahippocampal gyrus atrophy was not a significant predictor in analyses that controlled for demographic and cognitive variables.[26] One possible explanation is that the parahippocampal gyrus is relatively large, and regions other than its component entorhinal cortex (layer II is particularly vulnerable) may be relatively spared in early AD. Some of the boundaries in the entorhinal region of the parahippocampal gyrus can be difficult to define, increasing variability. Also, the parahippocampal gyrus volume included white matter, and the degree to which tissue loss extends into the white matter in early AD is uncertain.[27,28]

Older subjects had smaller hippocampal and entorhinal cortex volumes. Hippocampal volume decreases with age, especially after 80 years,[29,30] but the effect of age on entorhinal cortex volume varies considerably across studies.[6,13,30] In autopsies of very mild AD patients who were aged 60 to 90 years, no significant entorhinal cortex neuronal loss was found.[23] In our study, age was a significant predictor by itself, but it did not add materially to the prediction obtained from the combination of hippocampal and entorhinal cortex volumes (table 3). Further, apolipoprotein E ε4 allele carrier status did not alter the predictive accuracy of these MRI volumetric measures.[5]

This study has limitations. First, the results can be applied only to outpatients presenting for evaluation of memory complaints, and not to the general population. To improve clinical relevance, the inclusion/exclusion criteria broadly identified cognitively impaired patients without dementia and without a specific identifiable cause, thereby increasing heterogeneity. Most patients also met Peterson guidelines for MCI[7,8] and the main findings held in the subsample with amnestic MCI, though to a lesser degree, suggesting that the MRI variables may be less useful predictors of conversion to AD in samples restricted to patients with amnestic MCI. Second, the primary outcome of AD was based on clinical diagnosis, not neuropathology, and this may have led to classification error. This limitation was tempered by using expert raters who used strict diagnostic methods. Nonetheless, patients currently classified as nonconverters may convert to AD with longer follow-up. Third, recruitment for control subjects was done mainly by advertisement, which is not as optimal as peer-nominated recruitment, e.g., family or friends referred to be controls by the patients with MCI themselves. The latter was attempted, though unsuccessfully, in most cases. However, control subjects' data did not figure in testing the study hypotheses.

Other structural MRI measures have been shown to discriminate AD from controls: ventricular volume,[14,21] whole brain volume,[14] CSF volume,[21] and corpus callosum area.[31] The predictive utility of these measures for MCI conversion to AD is inconsistent across studies,[14,28,32] and intracranial volume did not predict conversion to AD in our sample.

Serial MRI scans at annual or longer intervals show that reduction in brain regional volumes discriminates AD from healthy controls[4,33] and is associated with future cognitive decline in normal elderly subjects.[34] Serial images reduce measurement variability, increasing the likelihood of identifying differences over time. However, clinical practicality for serial MRI imaging is limited because of increased cost, complexity related to three-dimensional image coregistration needed to assess atrophy rates, and the clinical need to promptly establish the diagnosis. Voxel-based morphometry,[35] surface mapping of hippocampal subregions,[12] and diffusion-weighted imaging[36] are other MRI strategies that have been studied in small samples, and their predictive utility for MCI conversion to AD in large cohorts remains to be established. Further, their predictive accuracy compared to assessment of hippocampal and entorhinal cortex volume is unclear. In a recent cross-sectional study, older adults with cognitive

complaints and neuropsychological test performance that did not meet the MCI threshold had hippocampal volume loss intermediate between patients with amnestic MCI and healthy controls, and medial temporal lobe gray matter loss comparable to patients with amnestic MCI using voxel-based morphometry.[37] This suggests that medial temporal lobe atrophy may precede the clinical diagnosis of MCI, but longer term follow-up is needed to confirm this hypothesis.

Other markers, such as decreased temporoparietal metabolism with fluorodeoxyglucose positron emission tomography (PET)[38] and PET amyloid imaging[39,40] and increased levels of total tau and phosphorylated tau and decreased $A\beta_{42}$ in CSF, are known to distinguish patients with AD from controls.[41] However, their utility in predicting MCI conversion to AD and their value in such prediction over and above age and cognitive predictors are not well established. Of note, a recent report suggests that increased total tau and P-tau_{181} and decreased levels of $A\beta_{42}$ in CSF are strong predictors of MCI conversion to AD.[42]

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