

ORIGINAL ARTICLE

PET of Brain Amyloid and Tau in Mild Cognitive Impairment

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ABSTRACT

BACKGROUND

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Amyloid senile plaques and tau neurofibrillary tangles are neuropathological hallmarks of Alzheimer's disease that accumulate in the cortical regions of the brain in persons with mild cognitive impairment who are at risk for Alzheimer's disease. Noninvasive methods to detect these abnormal proteins are potentially useful in developing surrogate markers for drug discovery and diagnostics.

METHODS

We enrolled 83 volunteers with self-reported memory problems who had undergone neurologic and psychiatric evaluation and positron-emission tomography (PET). On the basis of cognitive testing, 25 volunteers were classified as having Alzheimer's disease, 28 as having mild cognitive impairment, and 30 as having no cognitive impairment (healthy controls). PET was performed after injection of 2-(1-{6-[(2-[F-18] fluoroethyl)(methyl)amino]-2-naphthyl}ethylidene)malononitrile (FDDNP), a molecule that binds to plaques and tangles in vitro. All subjects also underwent 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) PET, and 72 underwent magnetic resonance imaging (MRI).

RESULTS

Global values for FDDNP-PET binding (average of the values for the temporal, parietal, posterior cingulate, and frontal regions) were lower in the control group than in the group with mild cognitive impairment ($P < 0.001$), and the values for binding in the group with mild cognitive impairment were lower than in the group with Alzheimer's disease ($P < 0.001$). FDDNP-PET binding differentiated among the diagnostic groups better than did metabolism on FDG-PET or volume on MRI.

CONCLUSIONS

FDDNP-PET scanning can differentiate persons with mild cognitive impairment from those with Alzheimer's disease and those with no cognitive impairment. This technique is potentially useful as a noninvasive method to determine regional cerebral patterns of amyloid plaques and tau neurofibrillary tangles.

MILD COGNITIVE IMPAIRMENT IS A transitional stage between normal aging and Alzheimer's disease. A recent study suggests that the prevalence of mild cognitive impairment, characterized by a cognitive decline without impairment of the ability to carry out activities of daily living, is 19% among persons younger than 75 years of age and 29% among those 85 years of age or older.¹ Among persons with mild cognitive impairment, about 30% have amnesic mild cognitive impairment, characterized by abnormal memory for age but normal general cognitive functioning.^{2,3} Approximately 12% of patients with amnesic mild cognitive impairment have progression to Alzheimer's disease each year, and up to 80% have progression to Alzheimer's disease after 6 years.² Alzheimer's disease is also progressive, gradually resulting in cognitive impairments that leave patients completely dependent on others.

Neuropathological studies indicate that two proteins, β -amyloid (in senile plaques) and tau (in neurofibrillary tangles), accumulate abnormally in a predictable spatial pattern during aging and in Alzheimer's disease.^{4,5} These changes may begin even before the age of 30 years, and the prevalence of the lesions increases gradually with age. In patients with mild cognitive impairment, neurofibrillary tangles have been detected in the hippocampus and other medial temporal regions; as mild cognitive impairment progresses to Alzheimer's disease, these neurofibrillary tangles spread to the parietal and frontal neocortical areas of the brain. Neuritic plaques are rare in persons undergoing normal aging but in those with mild cognitive impairment, they begin to accumulate in the hippocampus and neocortex, where the plaques become more prevalent with the progression to Alzheimer's disease. The presence of high cerebral levels of amyloid senile plaques and neurofibrillary tangles is currently necessary for a diagnosis of definite Alzheimer's disease at autopsy.⁶

Treatments are being developed for Alzheimer's disease that are designed to prevent the accumulation of cerebral plaques and tangles or to disaggregate them once they are present. A noninvasive method of determining the regional cerebral patterns of these lesions would not only assist in early diagnosis of Alzheimer's disease but also facilitate monitoring of the efficacy of such treatments.

Until recently, plaques and tangles could be assessed only at autopsy or, rarely, on biopsy. Positron-emission tomography (PET) with the use of amyloid tracers has shown higher levels of cerebral amyloid in patients with dementia than in control subjects.^{7,8} For example, PET studies performed with the use of Pittsburgh Compound-B (PIB), an amyloid-binding radiotracer, have shown significantly greater cortical PIB retention in patients with Alzheimer's disease than in controls.⁷ We developed a small molecule, 2-(1-{6-[(2-[F-18]fluoroethyl)(methyl)amino]-2-naphthyl]ethylidene)malononitrile (FDDNP), for use as an *in vivo* chemical marker of cerebral amyloid and tau proteins. Initial studies have shown that PET scans show significantly higher values for FDDNP binding in the temporal, parietal, and frontal regions of the brain in patients with Alzheimer's disease than in older control subjects without cognitive impairment.⁹ Both FDDNP and its parent molecule, 2-(1-[6-(dimethylamino)-2-naphthyl]ethylidene)malononitrile, are fluorescent and provide clear *in vitro* visualization of plaques and tangles in specimens of brain tissue obtained on autopsy from patients with Alzheimer's disease and examined with a confocal fluorescence microscope.¹⁰

We used *in vivo* cerebral imaging of these abnormal protein aggregates to study persons with mild cognitive impairment or Alzheimer's disease. Previous neuropathological studies led us to hypothesize that the intensity of FDDNP binding in those with mild cognitive impairment would be intermediate between the intensity found in healthy controls and in persons with Alzheimer's disease, and that in persons whose cognitive impairment progressed clinically, there would be corresponding increases in FDDNP binding.

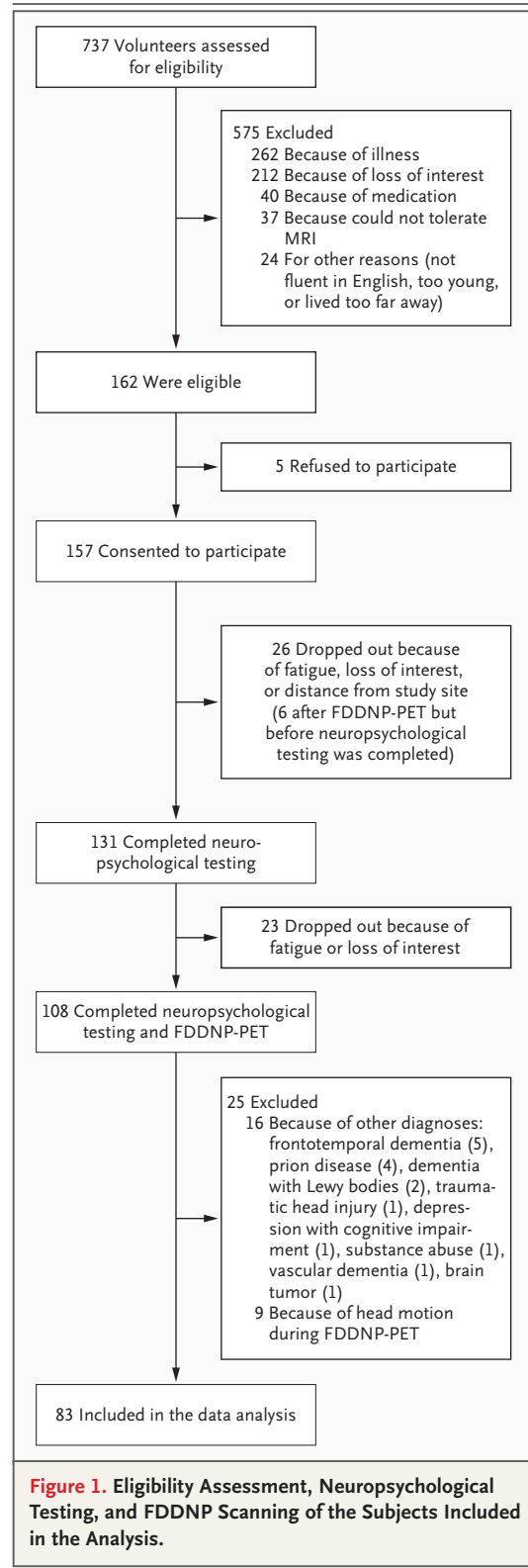
METHODS

CLINICAL ASSESSMENTS

Baseline cognitive assessments and PET were performed in 108 persons selected from a pool of 737 volunteers who were middle-aged or older (range, 49 to 84 years). The pool of volunteers had been recruited through advertisements of a study of mild memory impairment, media coverage of the study, and referrals by physicians and families and had been screened in telephone interviews conducted by members of the research staff. The study protocol described in detail the methods,

procedures, and prespecified inclusion and exclusion criteria. Because the study focused on Alzheimer's disease, subjects in whom other types of dementia had been diagnosed (e.g., Lewy body, vascular, or frontotemporal dementia) were excluded. From the original pool of volunteers, 40 persons taking medications that might affect cognition, such as sedatives, or nonsteroidal anti-inflammatory drugs, which bind to amyloid plaques and thus can affect FDDNP binding values, were excluded.¹¹ Others were excluded for a variety of reasons, including 9 because of head movement during scanning (6 with Alzheimer's disease, 1 with mild cognitive impairment, and 2 potential controls), resulting in a total of 83 subjects in the study sample (Fig. 1). Investigators were unaware of the clinical data when excluding potential subjects on the basis of the quality of the PET scans and were unaware of the scans when excluding potential subjects on the basis of the clinical data. Of the 83 subjects included in the study, 10 with Alzheimer's disease and 6 with mild cognitive impairment had been receiving drugs to enhance cognitive performance (a cholinesterase inhibitor or an N-methyl-D-aspartate receptor antagonist) at a steady dose for at least 3 months before entering the study.

All subjects underwent neurologic and psychiatric evaluation, screening laboratory testing, and structural imaging scanning to rule out other causes of cognitive impairment (e.g., stroke or tumor).⁶ Most of the subjects (72) underwent magnetic resonance imaging (MRI); the 11 subjects who could not tolerate MRI (because of claustrophobia or metal in the body) underwent computed tomography (CT). In addition to evaluation according to the Mini-Mental State Examination¹² and the 17-item Hamilton Rating Scale for Depression,¹³ we administered a battery of neuropsychological tests¹⁴ to assess five cognitive domains: memory (Wechsler Memory Scale Logical Memory Test and Verbal Paired Associations II, Buschke-Fuld for Selective Reminding Test [Total Recall], and Rey-Osterreich Complex Figure Recall Test [Delayed Recall]), language (Boston Naming Test, Letter Fluency Test and Animal Naming Test), attention and speed of information processing (Trail Making Test A, Stroop Color Test [Kaplan version], and Digit Symbol Test, Wechsler Adult Intelligence Scale [WAIS]), executive functioning (Trail Making



Test B, Stroop interference [Kaplan version], and Wisconsin Card Sorting Test, Perseverative Errors), and visuospatial functioning (WAIS Block Design Test, Rey–Osterreich Complex Figure Test [copy], and Benton Visual Retention Test).

To diagnose mild cognitive impairment, we used standard diagnostic criteria for amnesic mild cognitive impairment (memory impairment without other cognitive impairment). These include the subject's awareness of a memory problem, preferably as confirmed by another person; memory impairment detected with the use of standard assessment tests; normal overall thinking and reasoning skills; and the ability to perform normal activities of daily living.² For a broad definition of mild cognitive impairment, we also used guidelines to identify subjects with other subtypes of mild cognitive impairment, including memory impairment and additional cognitive deficits and those with other cognitive deficits but intact memory.¹⁵ The diagnosis was corroborated by clinical judgment²; to increase the specificity in detecting impairments, we included only subjects with mild cognitive impairment who had a score of 1 SD or more below the age-corrected norms on at least two neuropsychological tests in one of the five cognitive domains assessed.¹⁶

Subjects included in the group with mild cognitive impairment did not meet the diagnostic criteria for Alzheimer's disease,^{6,17} and the presence of self-reported memory problems was documented with the use of a standardized subjective-memory instrument (the Memory Functioning Questionnaire)¹⁸ and a clinical interview. Of the 28 subjects with mild cognitive impairment, 24 had memory impairment that was consistent with either amnesic mild cognitive impairment (9 subjects) or amnesic mild cognitive impairment plus deficits in other cognitive areas (15 subjects), and 4 subjects had mild cognitive impairment without memory impairment (i.e., impairment only in other cognitive areas).

Subjects with Alzheimer's disease met the standard diagnostic criteria of memory impairment, impairment in at least one other cognitive domain, gradual onset and progressive decline, and impaired occupational or social functioning or both.^{6,17} Control subjects had normal cognitive functioning for their age and did not meet the criteria for mild cognitive impairment or Alzheimer's disease. Follow-up clinical evaluations

and FDDNP-PET scans were available for 12 subjects (8 control subjects and 4 subjects with mild cognitive impairment) after approximately 2 years (mean, 24.3±6.1 months; range, 17 to 34). Of the 71 subjects not included in the follow-up, 37 had not participated in the study long enough and 34 had not completed the study (i.e., had moved, were unable to complete it, or had declined).

All clinical assessments and scanning procedures were performed within 4 weeks after study entry, and clinicians were unaware of the results of the baseline and follow-up studies. Written informed consent was obtained from all subjects or from a family member or guardian, in accordance with procedures of the Human Subjects Protection Committee of the University of California, Los Angeles. Cumulative radiation dosimetry for all scans was below the mandated maximum annual dose and in compliance with state and federal regulations. Four subjects had minor adverse events during PET: two subjects (one with Alzheimer's disease and one control) had minor bruising at venipuncture sites, and two others (one with Alzheimer's disease and one control) had transient headache.

SCANNING AND IMAGING PROCEDURES

The FDDNP was prepared at very high specific activities (>37 GBq per micromole), as described elsewhere.^{9,19} PET was performed with the ECAT HR or ECAT EXACT HR+ scanner (Siemens CTI), with subjects in the supine position and with the imaging plane parallel to the orbitomeatal line. A bolus of FDDNP (320 to 550 MBq) was injected through an indwelling venous catheter, and consecutive dynamic PET scans were obtained for 2 consecutive hours. Scans were corrected for decay and reconstructed with the use of filtered back-projection (Hann filter, 5.5 mm full width at half maximum), with correction for scatter and measured attenuation. The resulting images contained either 47 contiguous slices with a plane separation of 3.37 mm (with the ECAT HR) or 63 contiguous slices with a plane separation of 2.42 mm (with the EXACT HR+). The results did not differ significantly according to the scanner used.

Quantification of the data on FDDNP binding was performed with the Logan graphic method, with the cerebellum as the reference region for time points between 30 and 125 minutes.^{19,20} The slope of the linear portion of the Logan plot is

the relative distribution volume (DVR), which is equal to the distribution volume of the tracer in a region of interest (ROI) divided by the distribution volume of the tracer in the reference region. The DVR parametric images (incorporating radioactivity levels measured during the 2-hour PET scanning) were generated and analyzed with the use of ROIs drawn on the coregistered MRI or CT scans for left and right parietal, medial temporal (limbic regions, including the hippocampus, parahippocampal areas, and entorhinal cortex), lateral temporal, posterior cingulate, and frontal regions, as previously described.¹⁹ Each regional DVR or binding value was expressed as an average of the left and right regions, and global DVR values were calculated as averages of the values for all these regions. Rules for ROI drawing were based on the identification of gyral and sulcal landmarks with respect to the atlas of Talairach and Tounoux.²¹

Scans of the same subjects repeated within 2 weeks after the initial scans indicated the stability of these values (approximately 2.2% of the regional values). Quantification of the amount of radiotracer uptake for FDG-PET was performed on summed images (within 30 to 60 minutes after the radiotracer had been injected), and ROIs were drawn, as previously described,^{19,22} on regions that are sensitive indicators of early neurodegeneration (the parietal and posterior cingulate regions)^{22,23}; global values (the average of all ROIs) were also calculated. Values for ROIs were normalized to the value for the motor cortex.

Among the 72 subjects who underwent MRI, anatomical brain scans were obtained with the use of either a 1.5T (Signa) or 3T (General Electric or Siemens) scanner. For each of these subjects, 54 transverse planes were collected throughout the brain, superior to the cerebellum, with the use of a double-echo, fast spin-echo series with a 24-cm field of view and 256 × 256 matrix and 3-mm slices with no gap (repetition time, 6000 [3 T] or 2000 [1.5 T]; echo time, 17/85 [3 T] or 30/90 [1.5 T]). ROIs were drawn manually, as previously described,²⁴ on the right and left medial temporal lobes (the entorhinal cortex, hippocampus, and parahippocampal gyrus) and the ventricles. For comparisons among the three diagnostic groups, we used whole medial temporal (right plus left) volumes and ventricular volumes, regions found to be sensitive indicators of neurodegeneration.^{24,25} All PET and MRI scans were

read and the ROIs were drawn by investigators who were unaware of the clinical assessments.

NEUROPATHOLOGICAL PROCEDURES

Of the 83 study subjects, 1 subject died 14 months after baseline scanning, and a neuropathological evaluation was performed after autopsy. After the removal of portions of the fresh brain to be snap-frozen, the remainder of the brain was fixed in 10% neutral buffered formalin for approximately 10 days, then sliced coronally at intervals of 1 cm. A standard brain-blocking protocol²⁶ was carried out on the fixed slices, yielding representative fragments of all lobes of the cerebral hemispheres and the cingulate gyrus, the deep central gray matter, brain stem, and cerebellum. Representative sections were immunostained with primary antibodies to β -amyloid protein (1 to 42 amino acids in length) and to phosphorylated tau, with the use of commercially available antibodies routinely used in our laboratory.

STATISTICAL ANALYSIS

We used analysis of covariance to compare FDDNP binding values, FDG-PET metabolic rates, and MRI volumes among the three groups, controlling for age. We used receiver-operating-characteristic (ROC) curves to analyze the values measured with each type of imaging in order to determine the sensitivity, specificity, and area under the ROC curve (AUC). To determine relationships between values for FDDNP binding and other imaging values or between FDDNP binding and subjects' scores on cognitive testing, we used the Spearman rank correlation (r_s). The analysis was performed with the use of SAS software (version 9.1) and macros (ROC, ROCPLOT) available for use with the software. All reported P values are two-sided.

RESULTS

GROUP COMPARISONS

Of the 83 subjects in our sample, on the basis of cognitive testing, 25 were classified as having Alzheimer's disease, 28 as having mild cognitive impairment, and 30 as healthy controls. The three groups were similar with respect to the number of years of education, the proportion of women, and the proportion of subjects with a family history of dementia, but the control subjects were significantly younger than those with mild cognitive impairment or Alzheimer's disease ($P=0.01$).

Table 1. Demographic and Clinical Characteristics and Mean Imaging Values, According to Diagnostic Group.*

Variable	Diagnostic Group			P Value†
	Control (N=30)	Mild Cognitive Impairment (N=28)	Alzheimer's Disease (N=25)	
Age — yr	64±15	70±12	73±9	0.01
Education — yr	17±3	16±3	16±4	0.41
Female sex — no. (%)	12 (40)	17 (61)	13 (52)	0.28
Family history of Alzheimer's disease — no. (%)	8 (27)	8 (29)	7 (28)	0.98
Scores on cognitive tests				
Mini-Mental State Examination‡	29.3±1.1	27.2±1.7	19.9±6.5	<0.001
Digit Symbol§	71.3±17.1	53.2±18.6	26.1±18.9	<0.001
Verbal Paired Associations¶	6.7±1.6	3.6±2.9	—	<0.001
Selective Reminding	8.9±2.8	3.9±3.5	—	<0.001
FDDNP binding (DVR)				
Global value	1.07±0.02	1.12±0.02	1.16±0.01	<0.001
Medial temporal region	1.11±0.03	1.16±0.04	1.19±0.03	<0.001
Lateral temporal region	1.07±0.03	1.12±0.04	1.16±0.03	<0.001
Parietal region	1.05±0.03	1.10±0.03	1.16±0.02	<0.001
Posterior cingulate region	1.09±0.04	1.13±0.05	1.19±0.03	<0.001
Frontal region	1.03±0.03	1.07±0.03	1.11±0.02	<0.001
FDG glucose metabolism (SUVR)				
Global value	0.86±0.05	0.81±0.05	0.72±0.07	<0.001
Posterior cingulate region	1.04±0.08	0.96±0.09	0.83±0.12	<0.001
Parietal region	0.86±0.06	0.81±0.07	0.71±0.11	<0.001
MRI volume (cc)**				
Medial temporal region	4796±717	4347±1320	3846±1041	0.07 (0.17)
Ventricular region	36,594±23,755	45,337±27,554	57,750±19,397	0.22 (0.10)

* Plus-minus values are means ±SD. Quantification of the data on FDDNP binding was performed with the Logan graphic method, with the cerebellum as the reference region for time points between 30 and 125 minutes.^{19,20} The slope of the linear portion of the Logan plot is the relative distribution volume (DVR), which is equal to the distribution volume of the tracer in a region of interest (ROI) divided by the distribution volume of the tracer in the reference region. SUVR denotes standard uptake value relative.

† P values were calculated by analysis of variance for age and education and by the chi-square test for sex and family history of Alzheimer's disease. P values were calculated by analysis of covariance for the results of cognitive testing and imaging and controlling for age.

‡ Scores range from 0 to 30, with higher scores indicating better cognitive functioning.

§ Average normal raw scores (±1 SD) for ages 70 to 74 years range from 34 to 67 with higher scores indicating better functioning.

¶ Scores range from 0 to 8, with higher scores indicating better cognitive functioning.

|| Because most of the subjects with Alzheimer's disease were too impaired to complete the cognitive testing, only control subjects and subjects with mild cognitive impairment were included in this analysis. The mean score according to age norms is 9.1±2.6 among subjects 70 to 79 years of age; scores on this test range from 0 to 12, with higher scores indicating better cognitive functioning.

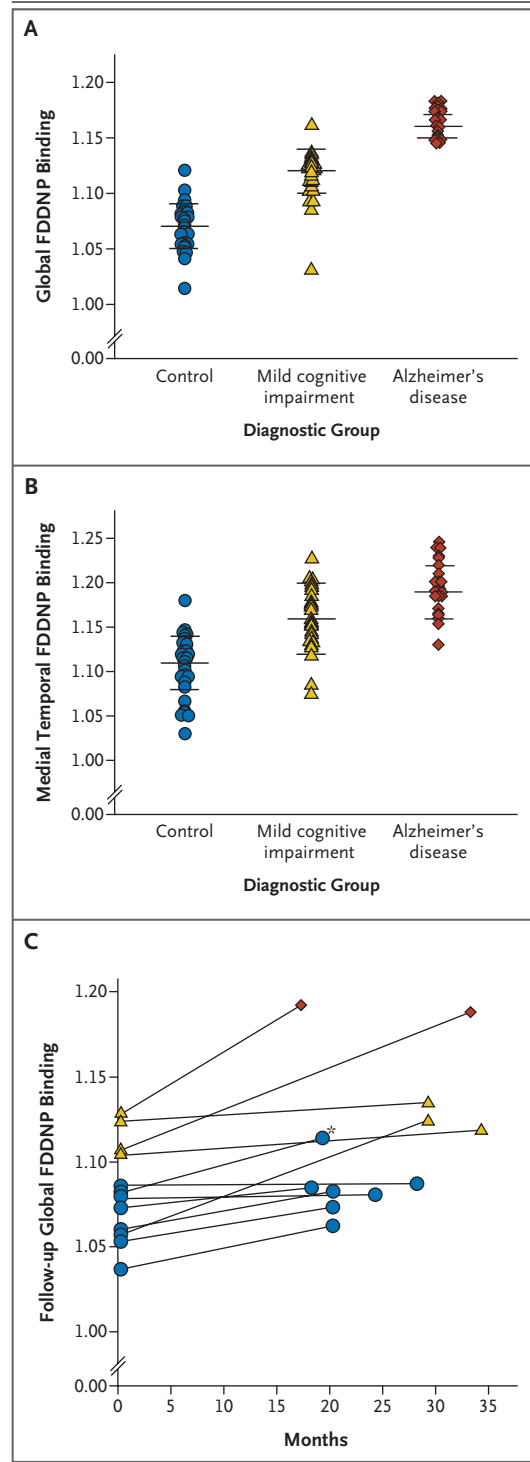
** MRI scans were not available for three control subjects, two subjects with mild cognitive impairment, and six subjects with Alzheimer's disease. P values in parentheses were obtained with the use of ratios (values for the medial temporal region or the ventricular region divided by the whole-brain volume) for between-group comparisons.

Figure 2. Baseline Values for FDDNP Global Binding (Panel A) and Medial Temporal Binding (Panel B), and Follow-up Values for FDDNP-PET (Panel C), According to Diagnostic Group.

Values for FDDNP global binding and medial temporal binding differed significantly among the three groups ($P < 0.001$). Although the condition of one control subject (indicated by the asterisk) did not meet the diagnostic criteria for mild cognitive impairment at follow-up, the subject's scores on five of seven memory subtests indicated a decline, and the score on the test for selective reminding was more than 1 SD below age-corrected norms. Long horizontal bars represent means, and short bars represent SDs. FDDNP binding is expressed in terms of the DVR derived by the Logan graphic method, with the cerebellum as the reference region.

Expected differences were also observed according to cognitive measures (Table 1). Mean (\pm SD) global values for FDDNP binding (DVR) differed significantly among the three groups: the values were significantly lower in the control group than in the group with mild cognitive impairment ($P < 0.001$), and the mean global values in the group with mild cognitive impairment were significantly lower than those in the group with Alzheimer's disease ($P < 0.001$) (Table 1 and Fig. 2). The estimated effect sizes (defined as the difference between the group means, divided by the pooled standard deviation) — 4.5 in the group with Alzheimer's disease as compared with the control group, 2.5 in the group with Alzheimer's disease as compared with the group with mild cognitive impairment, and 2.5 in the group with mild cognitive impairment as compared with control subjects — further demonstrated the robustness of global FDDNP binding for differentiating among the three diagnostic groups. The FDDNP-PET images in Figure 3 illustrate these differences.

Mean values for regional FDDNP binding in the temporal, parietal, posterior cingulate, and frontal regions were also significantly different among the three groups ($P < 0.001$) (Table 1). In all instances, the values for controls were significantly lower than those for subjects with mild cognitive impairment, and the values for subjects with mild cognitive impairment were significantly lower than those for subjects with Alzheimer's disease, with pairwise P values less than or equal to 0.001. Although the values for FDDNP binding in the medial temporal region differed significantly among the three groups (Table 1), 24 of the 28 subjects in the group with mild cognitive impairment had medial temporal FDDNP binding values of 1.13 or more, and 1.13 was the lowest



value in the group classified as having Alzheimer's disease (Fig. 2B).

In the ROC analysis comparing values for FDDNP-PET, FDG-PET, and MRI, FDDNP-PET global binding yielded the greatest diagnostic accuracy (Table 2). For the comparison between the

Figure 3. FDDNP-PET Scans at Baseline and Follow-up.

Panel A shows FDDNP-PET scans in the parietal region (top) and the temporal region (bottom) in one control subject and one subject with mild cognitive impairment who was reclassified on follow-up as having Alzheimer's disease. Scans of the subject with mild cognitive impairment, who was reclassified as having Alzheimer's disease, showed increased binding in the frontal (8.6%), parietal (8.9%), and lateral temporal (6.6%) regions. Red and yellow areas correspond to high FDDNP binding values. Panel B shows baseline MRI scans (top) and FDG-PET scans (bottom) of the same two subjects and scans of a third subject, with Alzheimer's disease, who died 14 months after undergoing baseline assessment. Red and yellow areas indicate high FDG uptake. In the control subject, the low value for FDDNP-PET binding is associated with minimal atrophy on MRI and high FDG uptake. Panel C shows the FDDNP-PET scan of the subject with Alzheimer's disease in Panel B and the results of the neuropathological microscopic examination after autopsy. Immunohistochemical staining was used to visualize β -amyloid protein (1 to 42 amino acids in length) and phosphorylated tau protein (both brown). Arrowheads indicate areas of higher power views (insets). The FDDNP-PET images are DVR parametric images (the DVR was derived by the Logan graphic method, with the cerebellum as the reference region). F denotes frontal, P parietal, PCG posterior cingulate, LT lateral temporal, and MT medial temporal.

group with Alzheimer's disease and the group with mild cognitive impairment, the AUC for FDDNP global binding (0.98; 95% confidence interval [CI], 0.95 to 1.00) was significantly greater than the AUC for FDG global metabolism (0.87; 95% CI, 0.77 to 0.97; $P=0.03$), FDG posterior cingulate metabolism (0.82; 95% CI, 0.71 to 0.94; $P=0.008$), FDG parietal metabolism (0.80; 95% CI, 0.67 to 0.92; $P=0.002$), or MRI medial temporal volume (0.62; 95% CI, 0.45 to 0.79; $P<0.001$). The comparison of the AUC between the FDG and MRI studies also showed a significant difference ($P=0.004$). For the comparison between the group with mild cognitive impairment and the control group, the AUC for FDDNP global binding (0.95; 95% CI, 0.87 to 1.00) was significantly greater than those for FDG global metabolism (0.77; 95% CI, 0.65 to 0.90; $P=0.009$), FDG posterior cingulate metabolism (0.74; 95% CI, 0.61 to 0.88; $P=0.006$), FDG parietal metabolism (0.70; 95% CI, 0.57 to 0.84; $P=0.001$), or MRI medial temporal volume (0.64; 95% CI, 0.48 to 0.80; $P<0.001$). The difference in the AUC between the FDG and MRI studies was not significant.

Among the subjects with mild cognitive impairment, comparisons of the global and regional

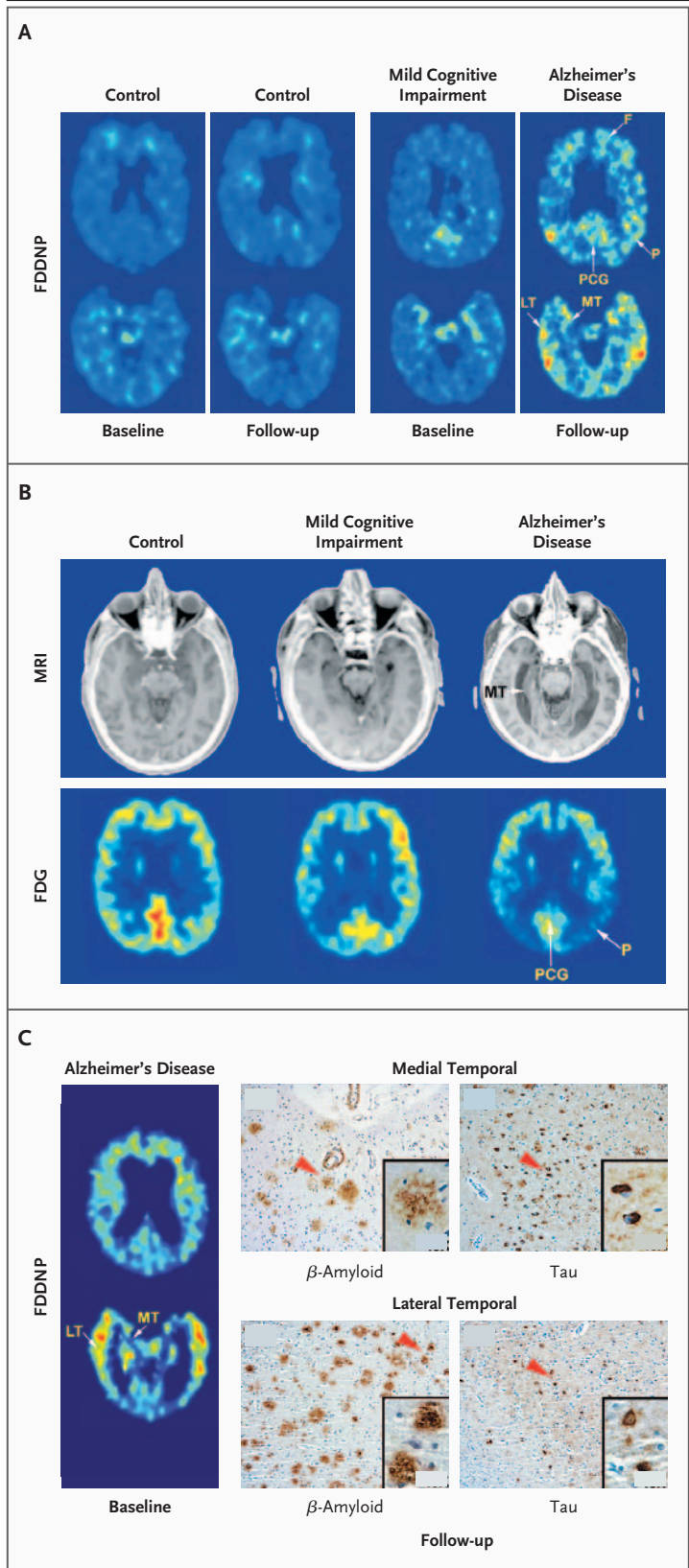


Table 2. Analysis of Receiver-Operating-Characteristic (ROC) Curves from Imaging Studies Performed with the Use of FDDNP-PET, FDG-PET, and MRI.

Imaging Study	Between-Group Comparisons		
	Alzheimer's Disease vs. Mild Cognitive Impairment	Mild Cognitive Impairment vs. Control	Alzheimer's Disease vs. Control
	area under ROC curve (95% CI)		
FDDNP-PET binding			
Global value*	0.98 (0.95–1.00)	0.95 (0.87–1.00)	1.00
Parietal region	0.93 (0.87–0.99)	0.88 (0.79–0.98)	1.00
Posterior cingulate region	0.86 (0.75–0.96)	0.75 (0.63–0.88)	1.00
Medial temporal region	0.75 (0.62–0.88)	0.88 (0.79–0.97)	0.98 (0.95–1.00)
FDG-PET metabolism (SUVr)†			
Global value*	0.87 (0.77–0.97)	0.77 (0.65–0.90)	0.94 (0.87–1.00)
Posterior cingulate region	0.82 (0.71–0.94)	0.74 (0.61–0.88)	0.93 (0.88–0.99)
Parietal region	0.80 (0.67–0.92)	0.70 (0.57–0.84)	0.89 (0.80–0.99)
MRI volume			
Medial temporal region*	0.62 (0.45–0.79)	0.64 (0.48–0.80)	0.80 (0.66–0.94)

* This value has the greatest diagnostic accuracy for this type of imaging.

† SUVr denotes standard uptake value relative.

FDDNP binding values between those with and those without memory impairment yielded no significant differences. There was no significant difference in the FDDNP binding values between the 10 subjects with Alzheimer's disease who were taking medications to enhance cognitive functioning (donepezil [Aricept, Pfizer] in 7 subjects and rivastigmine [Exelon, Novartis], memantine [Namenda, Forest], and donepezil plus memantine in 1 subject each) and those with dementia who were not taking such medications. In the group with mild cognitive impairment, there was no significant difference in the FDDNP binding values between the six subjects taking donepezil and those who were not taking medication to enhance cognitive functioning.

CORRELATIONS BETWEEN FDDNP-PET BINDING AND OTHER VARIABLES

As expected, higher values for global FDDNP binding correlated with lower values for FDG-PET in the posterior cingulate region (r_s , -0.64 ; $P < 0.001$) and the parietal region (r_s , -0.62 ; $P < 0.001$). Higher values for global FDDNP binding correlated significantly with lower MRI medial temporal volumes (r_s , -0.28 ; $P = 0.02$) and greater ventricular volumes (r_s , 0.36 ; $P = 0.002$).

Lower values for global FDDNP binding showed a strong correlation with higher scores on the

Mini-Mental State Examination (r_s , -0.75 ; $P < 0.001$) and the Digit Symbol test, Wechsler Adult Intelligence Test, third edition (r_s , -0.65 ; $P < 0.001$). Among the subjects with mild cognitive impairment and control subjects who completed other, more challenging cognitive tests, values for global FDDNP binding showed significant correlations with results on the Wechsler Memory Scale Verbal Paired Associations II (r_s , -0.57 ; $P < 0.001$) and the Buschke-Fuld Selective Reminding Test (Total Recall) (r_s , -0.52 ; $P < 0.001$).

SUBGROUP WITH LONGITUDINAL FOLLOW-UP

Longitudinal clinical and FDDNP-PET follow-up for approximately 2 years (mean, 24.3 ± 6.1 months; range, 17 to 34) indicated that the nine clinically stable subjects (seven controls and two with mild cognitive impairment) had only minimal increases ($\leq 3\%$) in global FDDNP binding. By contrast, the three subjects in whom there was clinical evidence of disease progression (one control subject was reclassified as having mild cognitive impairment, and two subjects with mild cognitive impairment were reclassified as having Alzheimer's disease) had greater increases in FDDNP binding, ranging from 5.5% to 11.2% (Fig. 2C and Fig. 3).

One subject with Alzheimer's disease (age, 78 years) died 14 months after the baseline clinical evaluation and FDDNP-PET scanning. Neuropatho-

logical studies of brain sections from this subject showed that regions with high in vitro concentrations of plaques and tangles closely matched those that showed increased in vivo FDDNP-PET binding (Fig. 3C). The medial temporal regions (hippocampus and entorhinal cortex) with high values for FDDNP binding showed abundant immunoreactive tangles but less abundant plaques, whereas other neocortical regions (the lateral temporal, posterior cingulate, and frontal regions) showed high concentrations of immunoreactive plaques, as well as some tangles.

DISCUSSION

The results of our study indicate that FDDNP-PET can differentiate mild cognitive impairment from normal aging and Alzheimer's disease. Moreover, evaluation at autopsy of one subject showed that regions of the brain with high values of FDDNP binding are characterized by high concentrations of plaques and tangles. These findings support the potential usefulness of FDDNP-PET in the development of surrogate markers for drug discovery aimed at blocking amyloid buildup and as a diagnostic tool, although the study does not provide definitive evidence of a basis for such uses. Because FDDNP-PET binding differentiates among clinical entities with varying severity of cognitive decline, it may eventually prove to be useful in the early detection of neurodegeneration.

The regional pattern of FDDNP binding that we observed was consistent with the patterns of accumulation of plaques and tangles observed in autopsy studies. Previous neuropathological studies in subjects with amnesic mild cognitive impairment²⁷ showed concentrations of medial temporal tangles that were intermediate in amount between those that occur in normal aging and those that occur in Alzheimer's disease, as well as widely distributed neuritic and diffuse plaques and tangles throughout the neocortex and limbic structures. Price and Morris⁵ noted that the spatial pattern and the progression of the accumulation of abnormal protein are consistent with changes in the interaction between plaques and tangles, in which at some point β -amyloid peptides cause an acceleration in the accumulation of age-related tangles that would otherwise accumulate relatively slowly with age. Tangle load, but not plaque load, is associated with cognitive decline in elderly persons.²⁷ Because FDDNP binds

to both plaques and tangles, the regional binding patterns may be helpful in differentiating between early Alzheimer's disease and normal aging, nonamnesic mild cognitive impairment, and other forms of dementia. Initial FDDNP studies of frontotemporal dementia²⁸ show binding in frontal and temporal regions but not in parietal regions, suggesting that FDDNP labels regional tau tangles and thus differentiates frontotemporal dementia from Alzheimer's disease according to the binding patterns.

The diagnosis of mild cognitive impairment refers to a presumed underlying pathobiologic state,² but the course of the condition is heterogeneous. Several subtypes of mild cognitive impairment have been defined, including amnesic mild cognitive impairment, the progressive course of which suggests it is a prodrome or preclinical form of Alzheimer's disease.^{2,3,15,16,27} Among the subjects in our study, most of those with mild cognitive impairment also had memory impairment (with or without other cognitive changes), as well as high FDDNP binding in the medial temporal regions similar to that in subjects with Alzheimer's disease, a finding that is consistent with the previous observation that amnesic mild cognitive impairment is often a prodrome of Alzheimer's disease.

We found that the values of global FDDNP were more accurate than previously established sensitive measures for FDG-PET^{22,23,29} or volumetric MRI measures^{24,25,30} for diagnostic classification of subjects, suggesting that FDDNP may be useful in differentiating among Alzheimer's disease, mild cognitive impairment, and normal aging. In these comparisons, values for FDDNP-PET global binding were more effective in discriminating among diagnostic groups than FDG-PET metabolism in the posterior cingulate or parietal regions^{22,23} or MRI volumes of the medial temporal regions, which many clinicians currently rely on for diagnostic confirmation of Alzheimer's disease.²⁵ Further studies are needed to determine whether combining several informative imaging techniques will improve diagnostic accuracy and whether the benefits of using multiple scans outweigh the added costs.

Some methodologic issues require comment. Only approximately 10% of the volunteers initially screened were included in the study, so the sample may not be representative of the populations studied. Our recruitment method yielded

a sample of motivated, highly educated, physically healthy subjects concerned about age-related memory problems and could have resulted in higher values for FDDNP-PET binding: concern about memory problems could have been a subtle indication of presymptomatic disease in some control subjects. Despite this potential bias, the FDDNP-PET binding values in the control group were significantly lower than those in the group with mild cognitive impairment and the group with Alzheimer's disease. The healthy controls were significantly younger than the subjects with mild cognitive impairment and those with Alzheimer's disease; however, we corrected for age in the analyses, and the results were similar in additional analyses when subjects younger than 55 years of age were excluded, eliminating the significance of differences in age among the three study groups. Comparisons involving subjects between 80 and 90 years of age would be needed to determine whether our results hold true for elderly subjects with Alzheimer's disease. The group with Alzheimer's disease had relatively mild degrees of dementia — subjects with more advanced disease were more likely to have been excluded because of coexisting conditions (e.g., hypertension or diabetes) or an inability to tolerate the study procedures. Since we used a group with mild, rather than severe, dementia for comparison, our findings held up even when the clinical differences between the groups were relatively subtle.

The proportion of women among the three diagnostic groups differed, but the difference was not significant. Although sex differences in brain function³¹ and brain structure³² have been reported, we found no significant differences in values for FDDNP binding between women and men. Many other factors could influence the results of quantitative PET scanning in elderly persons, including variations in scanners, use of medication, and head motion during imaging studies.

In summary, the results of our study indicate that FDDNP-PET scans differentiate persons with mild cognitive impairment from those with Alzheimer's disease and those without cognitive impairment. Equally important, *in vivo* distributions of FDDNP in the brain follow patterns of pathological distribution seen at autopsy.^{4,5,27} These observations suggest that FDDNP-PET may be useful in the development of surrogate markers for monitoring the accumulation of these abnormal protein aggregates in the brain that are characteristic of Alzheimer's disease.

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The University of California, Los Angeles (UCLA), owns a U.S. patent, "Methods for Labeling β -Amyloid Plaques and Neurofibrillary Tangles" (6,274,119), that uses the approach outlined in this article and has been licensed to Siemens. The FDDNP synthesis was performed at the UCLA Cyclotron Laboratory under Dr. Satyamurthy's direction. Drs. Small, Huang, Cole, Satyamurthy, and Barrio, who are among the inventors, report receiving royalties and will receive royalties on future sales. Dr. Small reports receiving consulting fees, lecture fees, or both from Abbott, Brainstorming, Dakim, Eisai, Forest, the Memory Fitness Institute, Myriad Genetics, Novartis, Ortho-McNeil, Pfizer, Radica, and Siemens, stock options from Dakim, and a grant from GlaxoSmithKline; Dr. Kepe, consulting fees from Siemens; Dr. Lavretsky, lecture fees from Eisai, Janssen, and Pfizer and a grant from Forest; Dr. Ercoli, lecture fees from the Memory Fitness Institute; Dr. Huang, lecture fees from GlaxoSmithKline; Dr. Satyamurthy, consulting fees from PETNet Pharmaceuticals and Siemens; and Dr. Barrio, consulting fees and lecture fees from Nihon Medi-Physics, Bristol-Myers Squibb, PETNet Pharmaceuticals, and Siemens. No other potential conflict of interest relevant to this article was reported.

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