Eur J Nucl Med Mol Imaging
DOI 10.1007/s00259-013-2458-z

ORIGINAL ARTICLE

Automated analysis of FDG PET as a tool for single-subject probabilistic prediction and detection of Alzheimer's disease dementia

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Received: 26 February 2013 / Accepted: 3 May 2013

Abstract
Purpose To introduce, evaluate and validate a voxel-based analysis method of ^{18}F-FDG PET imaging for determining the probability of Alzheimer's disease (AD) in a particular individual.
Methods The subject groups for model derivation comprised 80 healthy subjects (HS), 36 patients with mild cognitive impairment (MCI) who converted to AD dementia within 18 months, 85 non-converter MCI patients who did not convert within 24 months, and 67 AD dementia patients with baseline FDG PET scan were recruited from the AD Neuroimaging Initiative (ADNI) database. Additionally, baseline FDG PET scans from 20 HS, 27 MCI and 21 AD dementia patients from our institutional cohort were included for model validation. The analysis technique was designed on the basis of the AD-related hypometabolic convergence index adapted for our laboratory-specific context (AD-PET index), and combined in a multivariable

J. Arbizu and E. Prieto contributed equally to this study and should be considered equal first authors.

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

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Published online: 29 May 2013

model with age and gender for AD dementia detection (AD score). A logistic regression analysis of different cortical PET indexes and clinical variables was applied to search for relevant predictive factors to include in the multivariable model for the prediction of MCI conversion to AD dementia (AD-Conv score). The resultant scores were stratified into sixtiles for probabilistic diagnosis.

Results The area under the receiver operating characteristic curve (AUC) for the AD score detecting AD dementia in the ADNI database was 0.879, and the observed probability of AD dementia in the six defined groups ranged from 8 % to 100 % in a monotonic trend. For predicting MCI conversion to AD dementia, only the posterior cingulate index, Mini-Mental State Examination (MMSE) score and apolipoprotein E4 genotype (ApoE4) exhibited significant independent effects in the univariable and multivariable models. When only the latter two clinical variables were included in the model, the AUC was 0.742 (95 % CI 0.646 – 0.838), but this increased to 0.804 (95 % CI 0.714 – 0.894, bootstrap $p=0.027$) with the addition of the posterior cingulate index (AD-Conv score). Baseline clinical diagnosis of MCI showed 29.7 % of converters after 18 months. The observed probability of conversion in relation to baseline AD-Conv score was 75 % in the high probability group (sixtile 6), 34 % in the medium probability group (merged sixtiles 4 and 5), 20 % in the low probability group (sixtile 3) and 7.5 % in the very low probability group (merged sixtiles 1 and 2). In the validation population, the AD score reached an AUC of 0.948 (95 % CI 0.625 – 0.969) and the AD-Conv score reached 0.968 (95 % CI 0.908 – 1.000), with AD patients and MCI converters included in the highest probability categories.

Conclusion Posterior cingulate hypometabolism, when combined in a multivariable model with age and gender as well as MMSE score and ApoE4 data, improved the determination of the likelihood of patients with MCI converting to AD dementia compared with clinical variables alone. The probabilistic model described here provides a new tool that may aid in the clinical diagnosis of AD and MCI conversion.

Keywords FDG · PET · Alzheimer's disease · Mild cognitive impairment · Dementia · Prediction

Introduction

The diagnosis of Alzheimer's disease (AD) is predominantly clinical and is expressed in terms of probability. International working groups have recently revised the definition of AD by introducing a new lexicon and new diagnostic guidelines [1–3] emphasizing the concept that AD represents a *continuum*. Additionally, these criteria highlight the importance of in vivo biological evidence of AD pathology for enhancing the certainty of AD as the underlying cause of symptoms [4].

PET imaging using the most widely available radiotracer (^{18}F-fluorodeoxyglucose, FDG) has been established as a sensitive tool for detecting neuronal dysfunction in neocortical association areas. A recent review of the literature confirmed the increase in diagnostic accuracy obtained by using FDG PET in the evaluation of dementia, thus supporting its role as an effective and useful complementary tool for the early and differential diagnosis of AD [5]. Furthermore, FDG PET has been shown to be superior to other potential predictors of decline in patients with mild cognitive impairment (MCI) over a follow-up period of approximately 2 years [6]. Nevertheless, international working groups concur that much additional work is required to validate the incorporation of imaging biomarkers, such as FDG PET, in the diagnostic paradigm for symptomatic patients (MCI and AD). Part of this extensive work on biomarker standardization should include an agreement on how to obtain reproducible and objective results from PET images.

Standard clinical practice in brain FDG PET imaging is qualitative in nature. However, visual ratings depend heavily on the observer's prior experience, and therefore are subject to interobserver variability. Moreover, mild hypometabolism in small areas, such as the posterior cingulate or medial temporal cortex, can be difficult to evaluate without complementary quantitative analytical techniques. As a result, a strong effort has been made in this field over the last few years with respect to the development of different objective automated quantitative methods for FDG PET evaluation. Accordingly, several global indices of AD-related hypometabolism based on voxel-by-voxel analysis have been described. Three-dimensional stereotactic surface projection and NEUROSTAT-based indices [7], PMOD (PMOD Technologies) Alzheimer discrimination analysis tool (module PALZ) [8], meta-ROI-based metabolism computation (metaROI) [9], the AD-related hypometabolic convergence index (HCI) [10], and more recently, independent component analysis [11] are some of the available techniques.

The diagnostic performance of three of these global indices (PALZ, metaROI and HCI) has been directly compared in several independent groups of patients at different stages of AD taken from the largest FDG PET datasets currently available [12]. Nevertheless, no global index can be defined as the best-performing. On the other hand, these indices have been dichotomized for classification purposes [9, 10, 12, 13], but it is well known that the severity of an abnormal result may affect the likelihood or prognosis of AD [3].

Within this framework, the aim of the present study was to introduce an automated voxel-based analytical method involving FDG PET imaging that would determine the likelihood of AD in an individual, and the probability of conversion of MCI to dementia in a particular individual.

Specifically, we sought to identify cerebral metabolic abnormalities, along with demographic and clinical variables at baseline in MCI patients that, combined in a multivariable model, could be relevant predictive factors for conversion to clinically probable AD. The diagnostic performance of this technique was evaluated using the dataset of the Alzheimer's Disease Neuroimaging Initiative (ADNI), and validated using a cohort of patients and controls recruited at our institution.

Materials and methods

Patients for model derivation

The data used in the preparation of this article were obtained from the ADNI database (http://adni.loni.ucla.edu). The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and nonprofit organizations, as a $60 million, 5-year public/private partnership. The primary goal of ADNI has been to test whether serial MRI, PET, other biological markers, and clinical and neuropsychological assessments can be combined to measure the progression of MCI and early AD. Determination of sensitive and specific markers in very early AD progression is intended to aid researchers and clinicians develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials.

The Principal Investigator of this initiative is Michael W. Weiner, MD, of the VA Medical Center and University of California–San Francisco. ADNI is the result of the efforts of many coinvestigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the USA and Canada. The initial goal of ADNI was to recruit 800 adults, aged 55 to 90 years, to participate in the research, consisting of approximately 200 cognitively normal older individuals to be followed for 3 years, 400 individuals with MCI to be followed for 3 years, and 200 individuals with early AD to be followed for 2 years. For up-to-date information, see www.adni-info.org.

PET scans as well as clinical and demographic data of ADNI participants with a baseline FDG PET scan available on the ADNI web site as of July 2010 were considered for inclusion in this study. Among the whole dataset, the inclusion criteria for this study were: stable healthy subjects (HS), MCI and AD patients who maintained their clinical classification during a 24-month follow-up period, and MCI patients who converted to AD within an 18-month period (MCI-C). The AD and HS groups were randomly divided in two subgroups, paired by age and gender (AD-1, AD-2, HS-1, HS-2), for method implementation (creation of a map representing the hypometabolic pattern of AD; AD-1 and HS-1) and for performance evaluation (AD-2 and HS-2; see section PET analysis). Finally, 86 HS, and 121 MCI and 67 AD patients were included. The final groups for analysis and their demographic characteristics are shown in Table 1.

All the ADNI FDG PET images were acquired and reconstructed with measured-attenuation correction according to the standardized protocol for each tomograph model (http://adni.loni.ucla.edu/methods/documents/). Moreover, images were preprocessed by ADNI PET Coordinating Center investigators. Briefly, PET images acquired 30 to 60 min after injection were averaged, spatially aligned, interpolated to a standard voxel size, intensity-normalized, and smoothed to a common resolution of 8-mm full-width at half-maximum (FWHM).

Patients for model validation

An additional population was analysed to validate the proposed methodology. This population was recruited from patients at Clínica Universidad de Navarra and included 20 HS (vHS), 27 MCI patients (vMCI; six of whom converted within the 18-month follow-up, vMCI-C) and 21 AD patients (vAD). The demographic characteristics of these patients are included in Table 1. All subjects belonged to a larger cohort of patients and controls participating in a prospective longitudinal study on AD and MCI at the Memory Disorders Unit of the Clínica Universidad de Navarra. The Research Ethics Committee of this institution approved the study protocol, and written informed consent was obtained from all subjects.

All subjects included in this study received a thorough neurological evaluation, including a comprehensive neuropsychological evaluation and a full blood examination. Blood samples were drawn for ApoE analysis. ApoE was analysed by means of genotyping two single nucleotide polymorphisms (rs7412 and rs429358) with TaqMan® Gene Expression Assays as previously described [14]. The diagnosis of dementia was based on the DSM-IV criteria, and the diagnosis of probable AD was established according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (now known as the Alzheimer's Association) [15].

Patients with MCI were subjects with memory complaints who fulfilled Petersen Criteria for amnestic MCI [16]; that is, a clinical history from the subject, a reliable informant who confirmed abnormal cognitive deficits and memory performance below 1.5 standard deviations in at least one of the three administered verbal memory tests (delayed recall on the CERAD word-learning test, total recall on the free and cued selective reminding test, delayed free recall of the first paragraph from the logical memory

Table 1 Patient groups and demographic characteristics

Population		N	Age (years)[b]	Gender (percent female)	Education (years)[b]	MMSE score[b]	ApoE4 (percent of patients)[a]
ADNI	HS-1	43	77 (62 – 86)	79.1	16 (2 – 20)	29 (26 – 30)	25.6
	HS-2	43	75 (63 – 86)	74.4	16 (10 – 20)	29 (25 – 30)	25.6
	MCI-NC	85	77 (65 – 87)	29.4	16 (7 – 20)	28 (24 – 30)	43.5
	MCI-C	36	77 (65 – 89)	41.7	16 (12 – 20)	27 (24 – 29)	66.6
	AD-1	34	76 (59 – 88)	64.7	16 (4 – 20)	23 (20 – 26)	76.5
	AD-2	33	74 (60 – 88)	72.7	16 (11 – 19)	24 (20 – 26)	66.7
Validation	vHS	20	67 (65 – 76)	40.0	8 (8 – 20)	30 (26 – 30)	0
	vMCI-NC	21	73 (65 – 82)	33.3	12 (7 – 20)	28 (24 – 30)	38.1
	vMCI-C	6	73 (68 – 81)	50.0	15.5 (8 – 20)	27 (25 – 28)	50.0
	vAD	21	75 (65 – 82)	61.9	12 (2 – 20)	22 (20 – 28)	60.0

[a] Presence of at least one ApoE4 allele

[b] Median (range)

part of the Weschler Memory Scale-revised). All MCI patients had a score of 0.5 on the Clinical Dementia Rating and normal scores on the Yesavage geriatric depression scale and the interview for deterioration in daily living activities in dementia (IDDD) scale. Controls were recruited through an invitation letter to the local association of former blood donors of Navarra. Control subjects were volunteers older than 65 years who had no cognitive complaints and scored above the age- and education-adjusted cut-off in all cognitive tests, including all the three mentioned verbal memory tests. Exclusion criteria included a diagnosis of major depression, previous history of neurological or psychiatric disorders or any condition affecting brain structure or function (e.g. stroke, head trauma, or hydrocephalus). From an initial sample of 105 HS who volunteered to participate, 53 fulfilled the inclusion criteria and the first 20 consecutively recruited controls had an FDG PET scan performed.

Additionally, all subjects underwent a brain MRI study (Symphony 1.5 T; Siemens), and subjects with scores equal to or higher than 6 on the Age-Related White Matter Changes Rating Scale of the European Task Force [17] were excluded from PET investigation.

Patients were imaged on an ECAT EXACT HR+ PET tomograph (CTI/Siemens). Static emission images of 20-min duration were acquired 40 min after injection of 5.3 MBq/kg of ^{18}F-FDG. For attenuation correction, a 5-min transmission scan was acquired after each emission study using three external rotating ^{68}Ge rod sources. Images were reconstructed by filtered back-projection using a Hanning filter (cut-off frequency 4.9 mm), a zoom of 2.5 and a matrix size of 128 × 128 [18].

PET analysis

From among previously published techniques for the automatic analysis of individual FDG PET studies, the HCI index described by Chen et al. [10] was selected for use here. However, some requirements for HCI computation, such as the Matlab script, the AD-related map, and the reference population required for HCI computation were not available. Consequently, the whole procedure was reproduced and customized in our laboratory as described below.

Both ADNI and validation images were processed using Statistical Parametric Mapping software (SPM8; Wellcome Department of Cognitive Neurology, Institute of Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm). Briefly, FDG PET images were spatially normalized (using a custom FDG template), intensity normalized to the pons region (predefined over Montreal Neurological Institute space) and spatially smoothed with a 3-D gaussian kernel with an 8-mm FWHM. Statistical maps were also obtained using the SPM8 package [19].

Map of cerebral hypometabolism in the AD group: AD PET pattern

The first step in the analysis was the creation of a map representing the hypometabolic pattern of AD. To this end, the groups AD-1 and HS-1 were compared using a two-sample Student's t test. Significance was set at $p<0.05$ after family-wise error correction, and an extent threshold was set at 2,000 voxels. This threshold was based on the p values and the T scores of the clusters highlighted by the SPM analysis in the whole brain and represented 1.3 % of the voxels included in the brain mask. The z-score map obtained is referred to hereafter as the AD pattern [19].

Computation of the individual AD PET index

The next step was the development of an index that would compute the hypometabolic pattern of each subject of interest.

For this purpose, a *z*-score map of individual hypometabolism was calculated via a two-sample Student's *t* test between each individual and the HS-1 reference group. A probability threshold of $p<0.05$ (uncorrected for multiple comparisons) was considered to indicate statistical significance and the extent threshold was set at 100 voxels.

A metric was then defined to measure the similarity between the individual hypometabolic maps and the AD pattern. The similarity between each individual *z*-score map and the AD pattern was determined via a voxel-by-voxel product. The summation across all the voxels in the brain divided by 1,000 was defined as the AD PET index, with the resulting single number capturing the extent to which a PET image manifests the AD pattern. The procedure is shown in Fig. 1. This methodology was used to calculate the AD PET index of each patient belonging to the HS-2 and AD-2 groups.

Statistical analysis

All analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL). Normality of the variables was tested by the Shapiro-Wilk method. Quantitative data are reported as means ± standard deviation or median (range), depending on the distribution of the data. A significance level of $p<0.05$ was set to indicate significance for all comparisons.

Multivariable model for the detection of AD: AD score

Using the ADNI population (HS-2 and AD-2), the information provided by PET was integrated into a multivariable model that included age and gender (Fig. 1). The robustness of the model was assessed using a bootstrap procedure ($k=100$ datasets). The logistic model calculated an individual score for each patient, called AD score, which represented the predicted probability of AD in an individual.

To estimate the discrimination power of this model for the presence or absence of AD, AD scores of AD-2 and HS-2 subjects were compared using a box plot and the nonparametric Mann-Whitney *U* test. A receiver operating characteristic (ROC) curve was computed, with the total area under the curve (AUC), its 95 % confidence interval (CI), and the sensitivity and specificity at the optimal cut-off according to the Youden index (maximum of [sensitivity + specificity − 1]) calculated as indicators of overall goodness of the model. From these data, AD scores were recoded into sixtiles and the actual observed percentage with AD in each category was determined.

Multivariable model for prediction of MCI progression to AD: AD-Conv score

The same diagnostic tool designed for the discrimination between AD and HS subjects was directly translated to the ADNI population of MCI patients in order to classify them into converters and nonconverters to the AD dementia stage. In a similar manner to the above analysis, information provided by the individual SPM analysis of the PET images in each MCI patient against the HS-1 population was integrated into a multivariable model that included age and gender. The discriminating power of that model was evaluated using the Mann-Whitney *U* test and the ROC curve of the predicted probabilities.

Some variations of the model were analysed to adapt the AD classifier for the prediction of MCI conversion to AD. The first variation considered was based on the fragmentation of the AD PET index into several indexes related to anatomical brain areas, according to the Automated Anatomical Labeling cortical map [20]. These volumes of interest were grouped into five areas: left parietal, right parietal, left temporal, right temporal and posterior cingulate. The second variation considered was the inclusion in the model of clinical variables

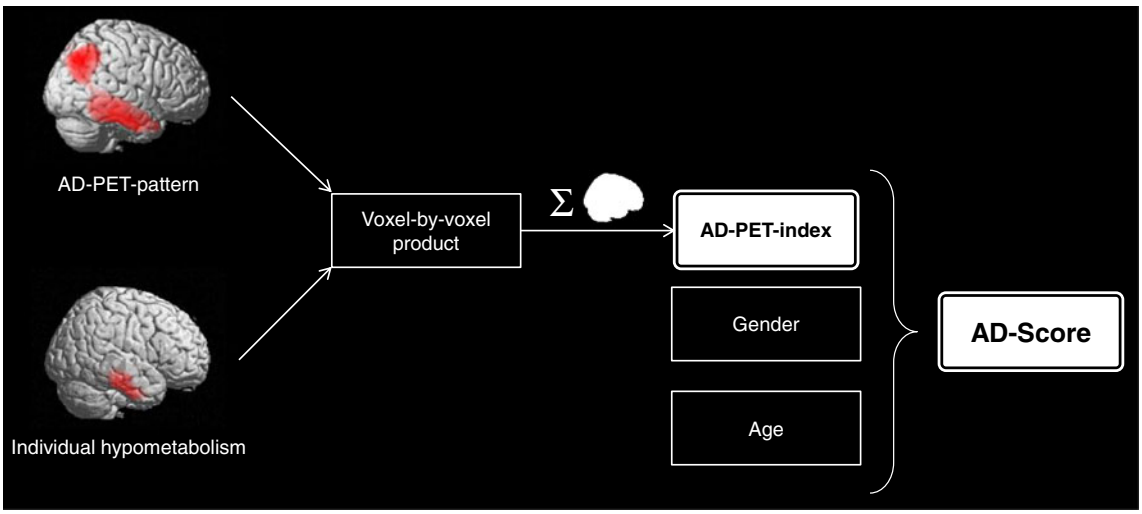

Fig. 1 Procedure for calculating the AD PET index and AD score

such as years of education, as well as the Mini-Mental State Examination (MMSE) score and apolipoprotein E (ApoE4) genotype (presence or absence of at least one ApoE4 allele). Statistical analyses were conducted to study which of these independent variables was especially relevant for discriminating MCI converters from nonconverters. For each possible independent variable considered (cortical regions and clinical variables), univariable and multivariable logistic regression was applied to search for relevant predictive factors. The criteria for possible inclusion in the multivariable model were a univariable p value <0.25 and a multivariable (adjusted) p value <0.05 [21]. The final model is constructed with the significant variables in the previous analysis adjusted for age and gender, with the obtained predicted probability labelled as AD-Conv score. The discriminating ability of this score, specifically adapted for predicting conversion from MCI to AD within 18 months, was evaluated using the Mann–Whitney U test and the AUC of the predicted probabilities. Paired AUC values from different possible models were compared using a bootstrapping procedure (k=100,000 samples) [22].

As with the AD model, AD-Conv scores were recoded from these data into sixtiles and the actual observed percentage of conversion to AD in each category was determined.

Prospective tool and validation of the proposed methodology

Models derived from the ADNI population, the AD score and the AD-Conv score were used to implement an automated calculation for prospective analysis (Microsoft Excel 2010). The calculation uses the estimated coefficients in the logistic model to determine the parameter score and to allocate the patient into a probability group according to the cut-off scores determined previously. The calculation was then used to evaluate each participant in the validation population. The AD score was determined for the vHS and vAD groups while the AD-Conv score was determined for the vMCI population, which included 22 vMCI-NC and 7 vMCI-C subjects. The AUCs for both diagnostic situations were estimated. Each patient was classified on the basis of his/her score calculated from the logistic model. The observed probabilities in the validation population were compared with the expected probabilities according to the ADNI cohort.

Results

AD PET pattern

Brain regions with a significant reduction in glucose metabolism in patients with AD compared with the those in the HS are shown in Fig. 2. Of particular note is the significant hypometabolism observed in the bilateral parietal cortex, the posterior cingulate, and the bilateral temporal cortex.

Multivariable model for detection of AD dementia: AD score

Patients with AD showed a median AD PET index of 78.7 and HS had a median value of 0.96. When age and gender were considered in the model, the median AD scores were 0.86 in AD patients and 0.18 in HS (Fig. 3). The Shapiro-Wilk test showed that normality was not fulfilled, while the Mann-Whitney U test showed statistically significant differences between the AD and HS groups ($p<0.001$). The AUC was 0.879 (95 % CI 0.797 – 0.962) (Fig. 3). The predicted probabilities generated with the bootstrapping procedure had a similar AUC (0.883, 95 % CI 0.787 – 0.958), thus attesting to the robustness of the model.

The optimal cut-off value calculated in terms of the maximum Youden index was 0.28 providing a sensitivity of 81.8 % (95 % CI 65.5 – 91.4 %), a specificity of 86.0 % (95 % CI 72.7 – 93.4 %), a positive predictive value of 82 % and a negative predictive value of 86 %. Several probability groups were defined instead of using dichotomized groups (HS and AD). The observed probability of a patient being correctly classified as AD was thus 8 % in the first sixtile, 15 % in the second, 23 % in the third, 38 % in the fourth, 77 % in the fifth, and 100 % in the sixth (Fig. 3).

Multivariable model for prediction of MCI progression to AD: AD-Conv score

The AUC for hypometabolism in all cortical regions was 0.739 (95 % CI 0.641 – 0.838). However, according to the univariable and multivariable models performed to select the best discriminant cortical regions within the AD pattern, only hypometabolism in the posterior cingulate area was significant in the multivariable model (Table 2). Moreover, among the clinical variables evaluated, ApoE4 genotype and MMSE score had a significant effect in both the univariable and multivariable models, while education level did not show a significant independent effect (Table 2).

When the MMSE score and ApoE4 genotype (age and gender) were considered in the model, the AUC was 0.742 (95 % CI 0.646 – 0.838). However, when posterior cingulate hypometabolism was also included in the model, the AUC improved significantly to 0.804 (95 % CI 0.714 – 0.894, bootstrap $p=0.027$,). Therefore, the final model for prediction of MCI conversion to AD dementia included hypometabolism in the posterior cingulate area together with the ApoE4 genotype and MMSE score (age and gender). The combination of these variables yielded a parameter called AD-Conv score. The procedure for calculating the discrimination score is shown in Fig. 4.

The optimal cut-off AD-Conv score to classify MCI patients into converters or nonconverters was 0.28, which yielded a sensitivity of 91.7 % (95 % CI 78.2 – 97.1 %), a

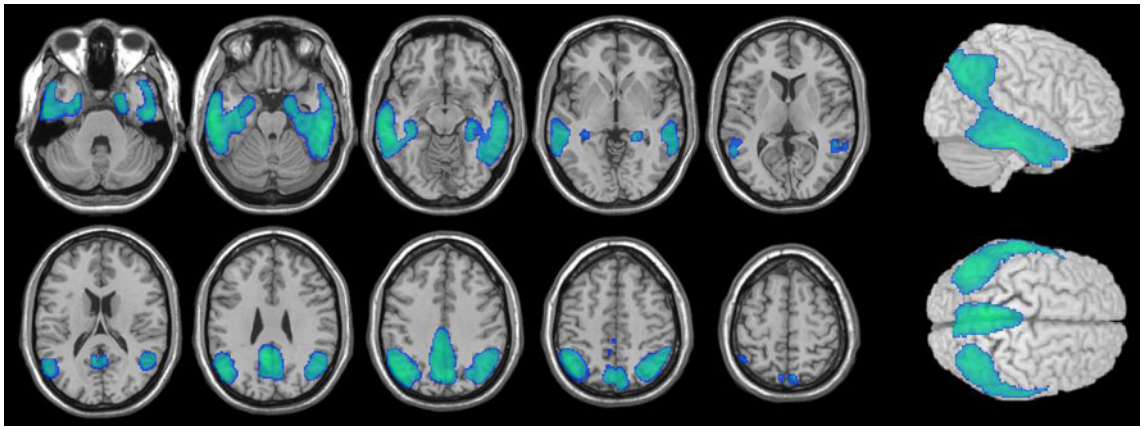

Fig. 2 Voxel-based analysis of group differences between the HS-1 and AD-1 groups from ADNI ($p<0.05$; family-wise error-corrected; 2,000 voxels). Overlain on MR images. Representative axial sections and render maps. The colour maps indicate the scale for the *T*-statistic

specificity of 62.4 % (95 % CI 51.7 – 71.9 %), a positive predictive value for conversion of 51 %, and a negative predictive value of 95 %.

In a similar manner to the AD protocol, classification in terms of risk group was preferred rather than the use of two dichotomized conditions. Due to the very similar probabilities in consecutive groups, the initial sixtiles of the distribution were merged into four categories called very low (first and second sixtiles), low (third and fourth sixtiles), medium (fifth sixtile) and high (sixth sixtile). The observed probabilities of conversion were 75 % in the high group, 34.1 % in the medium group, 20 % in the low group and 7.5 % in the very low group (Fig. 5).

Prospective tool and validation of the proposed methodology

A simple calculation was implemented for the prospective analysis and used for automated discrimination in the validation

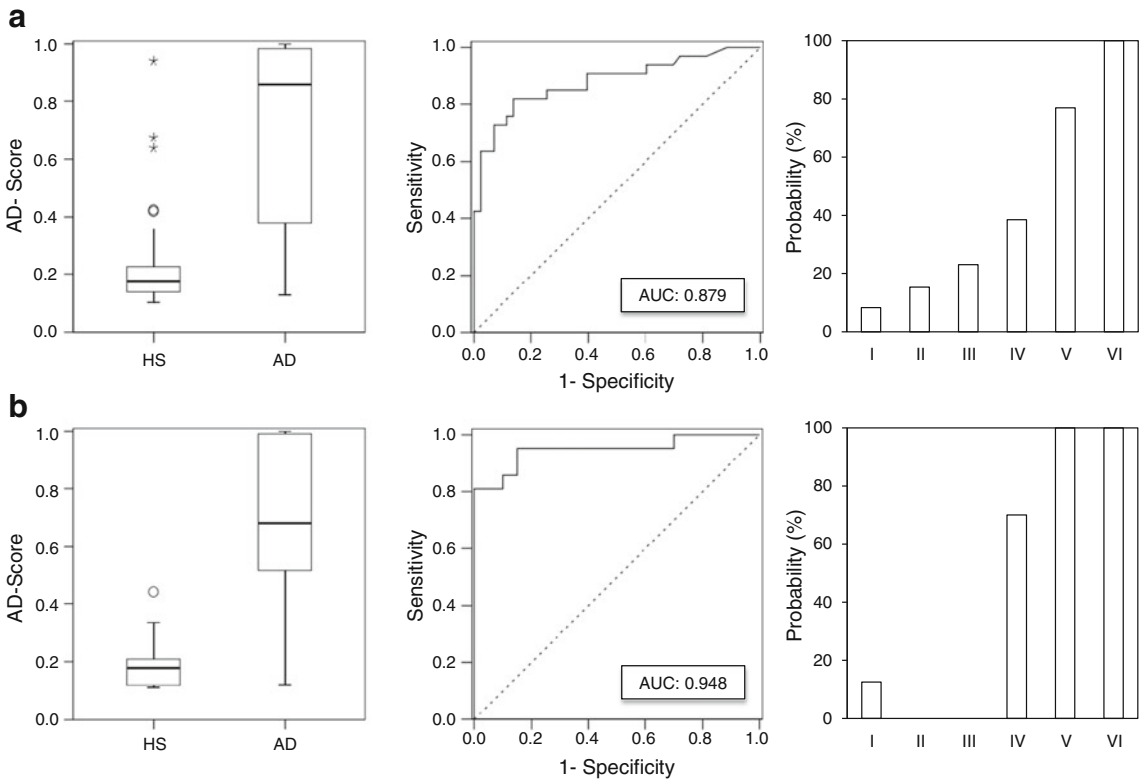

Fig. 3 Statistical results of the AD discrimination tool obtained for the derivation ADNI population (**a**), and the validation population (**b**). Box plots and ROC curves of the AD score to discriminate individually between AD patients and HS and the distribution of probabilities among sixtiles I to VI according to the score

Table 2 Results of the univariable and multivariable logistic regression analyses determining the independent variables relevant for MCI conversion discrimination

	Univariable p value	Multivariable[a] p value
Left parietal	0.002	0.810
Right parietal	0.004	0.651
Posterior cingulate	<0.001	0.026
Left temporal	0.001	0.410
Right temporal	0.005	0.877
ApoE4	0.022	0.021
MMSE score	0.005	0.030
Years of education	0.617	0.511

[a] Adjusted for gender and age

population. The median AD scores were 0.178 and 0.679 in the HS and AD patients, respectively ($p<0.001$, Mann-Whitney U test; Fig. 3). The AUC was 0.948 (95 % CI 0.625 – 0.969) and the observed probabilities were 10 % for the first sixtile, 0 % for the second and third sixtiles, 70 % for the fourth sixtile, and 100 % for the fifth and sixth sixtiles. All AD patients studied except one were assigned to a probability greater than 70 %.

On the other hand, the Mann-Whitney U test also demonstrated statistically significant differences in the AD-Conv scores between the MCI-NC and MCI-C patients (MCI-NC 0.161, MCI-C 0.451; $p<0.001$; Fig. 5). The AUC was 0.968 (95 % CI 0.908 – 1.000), and the probabilities of conversion were 100 % in the high group, 28.6 % in the medium group, and 0 % in both the low and very low groups. Therefore, for both discrimination tools, AD vs. HS and MCI-C vs. MCI-NC, the performance observed in the validation population was better than that in the ADNI cohort, thus confirming the power of these tools for use in different series of patients.

Discussion

In the present study, we defined and validated two probabilistic parameters obtained from an automated analysis of single-subject baseline FDG PET scans, these being the AD score and the AD-Conv score. The AD score measures the probability that a patient will suffer from AD dementia, and the AD-Conv score measures the probability that a patient with MCI will develop dementia due to AD within the next 18 months. Specifically, the combination of demographic data (age, gender) and clinical data (ApoE4 genotype, MMSE score) and posterior cingulate hypometabolism (AD-Conv score) yielded the best multivariable model for predicting which subjects with MCI would convert to AD.

In this study a probability or risk was calculated for each patient instead of a dichotomized result. In general, FDG PET global indices described in the literature classify each patient as "positive" or "negative" for AD according to a threshold value [9, 10, 13]. However, the application of a fixed cut-off to a biomarker continuum as represented by FDG PET can be questioned. In many patients, FDG PET quantification can be clearly normal (far below the threshold) or clearly abnormal (far above the threshold), but there is an intermediate range of values around the cut-off whose interpretation might be ambiguous [3]. Therefore, the assignation of probabilities is especially appropriate for these uncertain cases where the classification could better allocate individual patients into groups according to the likelihood of converting to AD (either AD dementia, or MCI conversion to AD dementia) associated with the hypometabolism observed in the PET image. This design has not been previously used and is well suited to the clinical complexity of AD, in which a prodromal phase precedes the development of dementia, and dementia severity progresses over time. The observed probability distribution pattern among

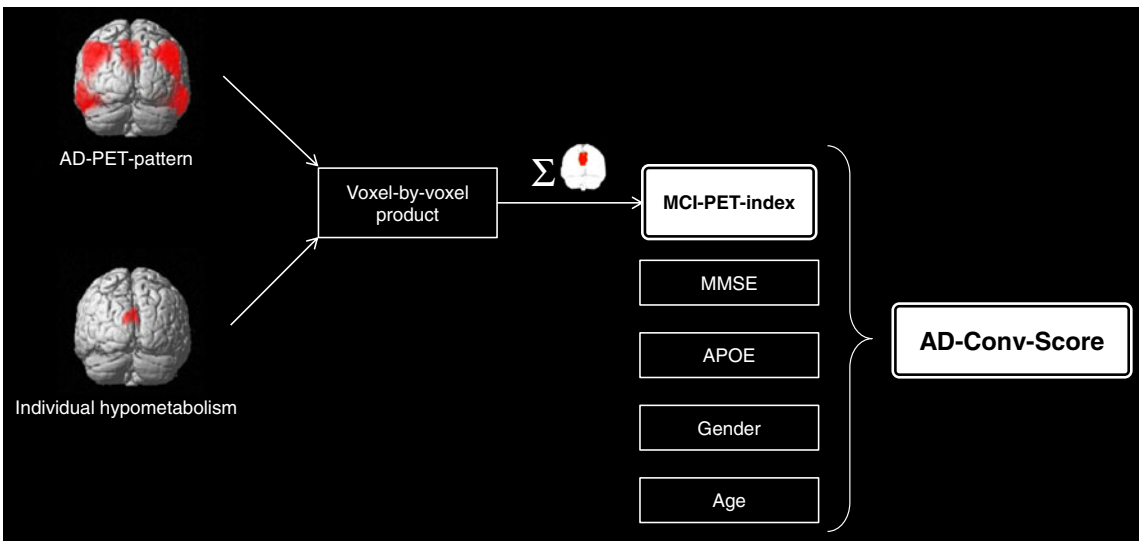

Fig. 4 Procedure for calculating the MCI PET index and AD-Conv score

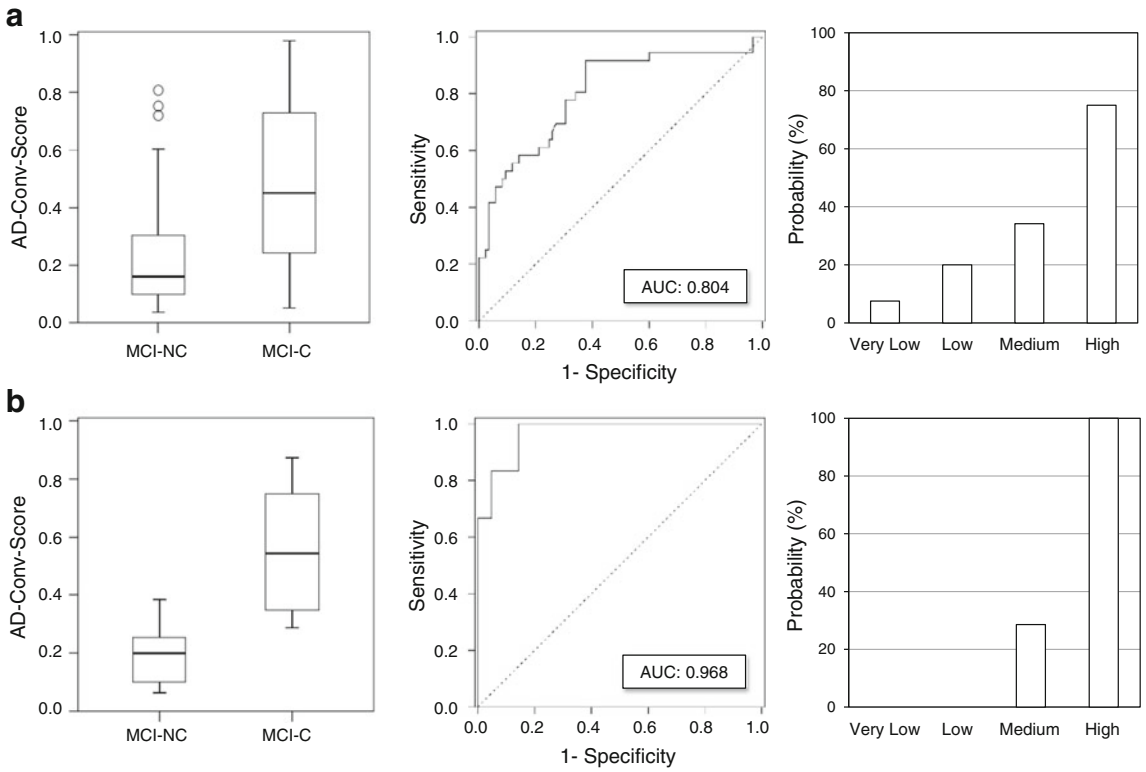

Fig. 5 Statistical results of the AD dementia conversion discrimination model obtained for the derivation ADNI population (**a**) and the validation population (**b**). Box plots and ROC curves of the AD-Conv score to discriminate individually between MCI converters and nonconverters and the distribution of probabilities among the stratified groups according to the score

the studied ADNI dataset confirms the continuous character of FDG PET as a biomarker of AD. Moreover, the distribution of probabilities of the AD-Conv score for predicting MCI conversion to AD dementia showed the complementary usefulness of this new tool. The baseline clinical diagnosis of MCI (using the strict criteria applied by ADNI) demonstrated a probability of conversion of 29.7 % (36 patients out of 121 MCI patients after 18 months). Therefore, the addition of baseline FDG PET data to clinical data would increase the probabilities to 75 % and 34.1 % in patients with a high risk and medium risk, respectively, according to the AD-Conv score. On the other hand, in patients with a low and very low risk the clinical probabilities of progression would decrease to 20 % and 7.5 %, respectively. An important advantage of this probabilistic detection of patients at different stages of prodromal AD could significantly improve the design of trials for new drugs targeting progression to dementia in subjects with MCI due to AD.

Detection of AD dementia (AD score)

Our study confirms the high diagnostic accuracy of FDG PET in detecting AD at the dementia stage. The AUC obtained using the ADNI population was 0.879 and the observed probability of AD dementia in the six groups ranged from 8 % to 100 % in a monotonic trend. Although a cut-off value was not used for the final diagnosis, sensitivity and specificity were computed for comparative purposes, with values of 81.8 % and 86 %, respectively. Similar results were obtained by Landau et al. using the metaROI global index in a similar ADNI population (AUC 0.88, sensitivity 82 %, specificity 70 %) [6].

The discrimination between AD patients and HS has been extensively studied, with a recently published review of related literature from 2001 to 2010 by Bohnen et al. [5]. According to this review of cross-sectional case-control studies, FDG PET revealed an overall sensitivity ranging from 80 % to 100 %, and a specificity ranging from 60 % to 100 % depending on the diagnostic reference standard used (clinical assessment, longitudinal clinical follow-up or post-mortem diagnosis). The AUC observed in the present work with the ADNI population was less than the 0.978 found by Caroli et al. [12] using an implementation of HCI, but similar to the 0.948 obtained in our validation population. Caroli et al. [12] found differences in all the FDG PET global metrics evaluated according to the dataset explored. Our methodology differed from the HCI in the selected population from the ADNI dataset and the AD pattern, which was computed in our imaging laboratory. Even though it was not the goal of this study to directly compare our results with those techniques described in the literature, differences in the number of subjects or in thresholding of the *z*-score maps might underlie the differences between our results and

those of others. However, the bootstrapping procedure performed in this study demonstrated the robustness of the model. Moreover, the AD score in our validation population showed that most of the AD patients had a 100 % probability of being correctly classified as AD. For further analysis, this tool is available and can be downloaded at: http://www.cun.es/la-clinica/servicios-medicos/departamento/medicina-nuclear/ad-and-mci-analysis-tool.

Prediction of MCI progression to AD dementia (AD-Conv score)

Different biomarkers or combinations of biomarkers have recently been studied with the aim of predicting cognitive decline or conversion to dementia in patients with MCI [6, 23, 24]. Predictors associated with conversion have been defined as those biomarkers that probably reflect disease severity or the proximity of an individual to a significant clinical transition [6]. In the logistic regression models performed in our study, ApoE4 genotype, MMSE score and FDG PET hypometabolism in the parietal, temporal and posterior cingulate areas were significant predictors of conversion in the univariable model. In the multivariable model predicting conversion, ApoE4, MMSE score and posterior cingulate hypometabolism had a significant independent effect.

A number of previous studies have shown that HS, and MCI and AD patients who are ApoE4 carriers have reduced cerebral glucose metabolic rates in the same brain regions, and ApoE4-carrier patients with probable AD have smaller hippocampal volumes and increased whole brain atrophy rates than noncarriers [25–28]. However, in our study, ApoE4 genotype and PET hypometabolism in the posterior cingulate cortex showed significant independent effects and consequently were included together in the model for prediction of MCI conversion to probable AD. Moreover, there was a significant improvement in performance when the posterior cingulate index was added to the model of clinical variables (ApoE4 and MMSE score). These results are consistent with those of studies examining the combination of FDG PET and ApoE4 genotyping in the early stratification of patients [29, 30]. Additionally, it has also been shown that MRI combined with ApoE4 genotyping is also useful for predicting conversion to AD [31].

It is not surprising that a measure of cognitive impairment could improve the performance of the model, as this has also been reported when cerebrospinal fluid biomarkers and volumetric MRI data are added to memory impairment [11, 32, 33]. Inclusion of the MMSE score was considered in the PALZ global index described by Herholz et al. [8], but in a different manner. The AD pattern from which the PALZ PET score was derived was identified in a cross-sectional sample by correlating hypometabolism in FDG PET images with the MMSE score, and therefore it was not directly added to the hypometabolic score (sensitivity 57 %, specificity 67 %, positive predictive value 45 %, negative predictive value 77 %, AUC 0.75). Contrary to our results, the addition of the MMSE score did not decrease the misclassification rate of MCI converters as was seen in the study by Shaffer et al. [11].

The posterior cingulate region is consistently described in the literature as an area of rapid change in the course of AD [34–37]. Therefore, this region has been consistently included in the set of derived ROIs reflecting an AD hypometabolism pattern and, more recently, in the FDG PET components extracted using independent principal component analysis to identify future decline in subjects with MCI [9]. However, the individual contribution of every single cortical region to predict the conversion to AD dementia in MCI patients from the ADNI dataset has not been explored. According to our results, it might be hypothesized that the hypometabolism in regions other than the posterior cingulate cortex do not have a significant independent effect and do not add more information to the model for the prediction of MCI conversion to AD. Moreover, it has also been reported that only posterior cingulate hypometabolism reaches significance when MCI patients who develop AD dementia after 1 year are compared to patients still classified as stable MCI [38]. In this respect, it is noteworthy that metabolic reductions in the posterior cingulate may be explained in part by the dysfunction of hippocampal output pathways due to AD pathology [39]. Additionally, Morbelli et al. [40] concluded that reduced metabolic connections both in hypometabolic and nonhypometabolic areas in patients with prodromal AD indicate that metabolic disconnection may predate remote hypometabolism as an early sign of synaptic degeneration.

Taking the hypometabolism of all cortical regions into account (global index; AUC 0.739), our results are consistent with those of previous studies showing the predictive value of FDG PET in the series of MCI patients from ADNI [6, 13]. However, these findings did not differ from those obtained when only clinical variables (MMSE score and ApoE4) were used in the multivariable model studied here (AUC 0.742). It is remarkable that addition of the posterior cingulate index to the clinical variables (AD-Conv score) significantly increased the AUC of the multivariable model to 0.804. Moreover, the AUC obtained in our prospective validation population reached 0.968, reflecting better predictive performance than in the ADNI population. One possible explanation for this finding may be related to the characteristics of the control group. This group in our validation sample was highly selected because only subjects with normal scores in three out of three verbal memory tests were recruited. These criteria may explain the surprisingly low proportion of ApoE4 carriers in this group. The inclusion of these "supernormal" controls may have contributed to improving the performance of the AD-Conv score in the validation process. Nevertheless, Caroli et al. found an AUC of 0.926 using the HCI global index [12] to compare MCI

converters and HS. Thus, a predictive biomarker of conversion to AD dementia should be tested on the entire population of MCI patients initially at risk and not only on those who finally convert.

Limitations

There were several limitations to this study that warrant comment. First, we included as a reference population the entire cohort of healthy controls available in the ADNI dataset in whom an FDG PET scan had been performed at baseline and who met the criteria described in the section Materials and methods (86 subjects). However, it is possible that a subgroup of HS positive in amyloid PET studies could have been included, and who consequently might have had preclinical AD. This could have affected the results by underestimating the performance of the method for predicting and detecting AD.

Second, the population studied here (especially MCI converters) consisted of a relatively small number of subjects and, therefore, the results obtained should be confirmed prospectively in a larger series of patients in a multicentre study. Nevertheless, the validation performed confirms the robustness and comparability of the FDG PET scores used as they worked even better in this population than in the model derivation series.

Third, the performance of the AD-Conv score in our study could have been limited by the addition of an overly general measure of cognitive impairment (MMSE score) to the model. As such, the model might benefit from the use of a more specific measure such as episodic memory. In this regard, it was shown that MCI patients with an abnormal FDG PET scan and low episodic memory performance on the auditory verbal learning test were almost 12 times more likely to convert to AD dementia than individuals who were normal on both measures [6].

Finally, we proposed a probabilistic method for the complementary diagnosis of clinical AD, but the clinical significance of categorizing patients with MCI as at medium or high risk of developing AD dementia, and the correlation of this categorization with time to conversion, has to be established.

Conclusion

Once a diagnosis of amnestic MCI is firmly established on clinical grounds and other aetiologies have been adequately ruled out, posterior cingulate hypometabolism, when combined in a multivariable model with age and gender as well as MMSE score and ApoE4 data, improved the determination of likelihood of patients with MCI converting to AD compared to clinical variables alone. In contrast to the interpretation of a biomarker as being positive or negative for AD, the probabilities rendered by the AD score and AD-Conv score are more appropriate for reflecting the slowly progressive nature of this pathology and the clinical manifestations of AD. Therefore, the proposed methodology could help to implement the new criteria for MCI due to AD set down in the National Institute on Aging/Reagan Institute of the Alzheimer Association Consensus Recommendations [3]. Moreover, this methodology provides the advantage of properly detecting patients at different stages of prodromal AD and as such could complement inclusion criteria for clinical trials.

Acknowledgments This work was supported in part by the Government of Spain, Institute of Health Carlos III, the Ministry of Health grant 01/0809, and the Ministry of Science and Innovation grant ADE 10/00028, and CB06/05/0077 CIBERNED (Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas).

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI; National Institutes of Health grant U01 AG024904). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: Abbott; Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Amorfix Life Sciences Ltd.; AstraZeneca; Bayer HealthCare; BioClinica, Inc.; Biogen Idec Inc.; Bristol-Myers Squibb Company; Eisai Inc.; Elan Pharmaceuticals Inc.; Eli Lilly and Company; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; GE Healthcare; Innogenetics, N.V.; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Medpace, Inc.; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Servier; Synarc Inc.; and Takeda Pharmaceutical Company. The Canadian Institutes of Health Research provides funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study was coordinated by the Alzheimer's Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuroimaging at the University of California, Los Angeles. This research was also supported by NIH grants P30 AG010129 and K01 AG030514.

Conflict of interest M.W. Weiner is the principal investigator of ADNI and declares the above-mentioned organizations as contributors to the Foundation for NIH and thus to the NIA-funded ADNI. The remaining authors have no conflicts of interest to declare.

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