

## MEDICAL SCIENCES

Correction for “Genetic inactivation of AKT1, AKT2, and PDPK1 in human colorectal cancer cells clarifies their roles in tumor growth regulation,” by Kajsa Ericson, Christine Gan, Ian Cheong, Carlo Rago, Yardena Samuels, Victor E. Velculescu, Kenneth W. Kinzler, David L. Huso, Bert Vogelstein, and Nickolas Papadopoulos, which appeared in issue 6, February 9, 2010, of *Proc Natl Acad Sci USA* (107:2598–2603; first published January 20, 2010; 10.1073/pnas.0914018107).

The authors note that all columns and error bars in their figures represent means and SDs.

[www.pnas.org/cgi/doi/10.1073/pnas.1002415107](http://www.pnas.org/cgi/doi/10.1073/pnas.1002415107)

## NEUROSCIENCE

Correction for “Subregional neuroanatomical change as a biomarker for Alzheimer’s disease,” by Dominic Holland, James B. Brewer, Donald J. Hagler, Christine Fenema-Notestine, Anders M. Dale, and the Alzheimer’s Disease Neuroimaging Initiative, which appeared in issue 49, December 8, 2009, of *Proc Natl Acad Sci USA* (106:20954–20959; first published November 20, 2009; 10.1073/pnas.0906053106).

The authors note that the author name Christine Fenema-Notestine should have appeared as Christine Fennema-Notestine. The corrected author line appears below. The online version has been corrected.

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**EDITORIAL EXPRESSION OF CONCERN.** PNAS is publishing an Editorial Expression of Concern regarding the following two articles:

**(i) BIOPHYSICS.** “Structure of vaccinia complement protein in complex with heparin and potential implications for complement regulation,” by Vannakambadi K. Ganesh, Scott A. Smith, Girish J. Kotwal, and Krishna H. M. Murthy, which appeared in issue 24, June 15, 2004, of *Proc Natl Acad Sci USA* (101:8924–8929; first published June 3, 2004; 10.1073/pnas.0400744101).

**(ii) BIOPHYSICS.** “Crystal structure of human apolipoprotein A-I: Insights into its protective effect against cardiovascular disease,” by A. Abdul Ajees, G. M. Anantharamaiah, Vinod K. Mishra, M. Mahmood Hussain, and H. M. Krishna Murthy, which appeared in issue 7, February 14, 2006, of *Proc Natl Acad Sci USA* (103: 2126–2131; first published February 1, 2006; 10.1073/pnas.0506877103).

The editors wish to note that we have received a report from the University of Alabama at Birmingham (UAB) that has investigated allegations of falsified or fabricated protein crystallographic structures including PDB codes 1RID and 2A01, which were published in the PNAS papers noted above. The UAB committee has forwarded their findings to the US Office of Research Integrity (ORI). We are awaiting the findings of ORI to determine the appropriate next steps.

Randy Schekman  
*Editor-in-Chief*

[www.pnas.org/cgi/doi/10.1073/pnas.1003210107](http://www.pnas.org/cgi/doi/10.1073/pnas.1003210107)

# Subregional neuroanatomical change as a biomarker for Alzheimer's disease

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Edited by Larry R. Squire, Veterans Affairs Medical Center, San Diego, CA, and approved October 1, 2009 (received for review June 2, 2009)

Regions of the temporal and parietal lobes are particularly damaged in Alzheimer's disease (AD), and this leads to a predictable pattern of brain atrophy. In vivo quantification of subregional atrophy, such as changes in cortical thickness or structure volume, could lead to improved diagnosis and better assessment of the neuroprotective effects of a therapy. Toward this end, we have developed a fast and robust method for accurately quantifying cerebral structural changes in several cortical and subcortical regions using serial MRI scans. In 169 healthy controls, 299 subjects with mild cognitive impairment (MCI), and 129 subjects with AD, we measured rates of subregional cerebral volume change for each cohort and performed power calculations to identify regions that would provide the most sensitive outcome measures in clinical trials of disease-modifying agents. Consistent with regional specificity of AD, temporal-lobe cortical regions showed the greatest disease-related changes and significantly outperformed any of the clinical or cognitive measures examined for both AD and MCI. Global measures of change in brain structure, including whole-brain and ventricular volumes, were also elevated in AD and MCI, but were less salient when compared to changes in normal subjects. Therefore, these biomarkers are less powerful for quantifying disease-modifying effects of compounds that target AD pathology. The findings indicate that regional temporal lobe cortical changes would have great utility as outcome measures in clinical trials and may also have utility in clinical practice for aiding early diagnosis of neurodegenerative disease.

clinical diagnosis | clinical trials | disease-specific atrophy | image registration | MRI biomarkers

The healthy adult brain is remarkably stable structurally but undergoes gradual changes with normal aging. Structural change is accelerated in neurodegenerative disease, including Alzheimer's disease (AD). The atrophy in AD arises from neuron and synapse loss that begins in the entorhinal cortex. The pathology then spreads throughout the limbic regions of the temporal lobe, including the hippocampal formation. Subsequently, neuron loss and atrophy is observed throughout neocortical association areas in temporal, parietal, and frontal lobes (1).

The fact that atrophy associated with AD can be detected in vivo using MRI has long been known (2). Hippocampal volume loss is a consistent finding (3) and is predictive of clinical decline (4–7). However, hippocampal atrophy is not specific to AD, as it is seen in a number of psychiatric and neurodegenerative diseases (8–10). Recently, it has been shown that cortical atrophy measured on MRI parallels the spread of AD pathology (11–13). Accurate measurement of cortical thickness and subcortical volumes across multiple regions may provide a signature of the disease specific enough to be useful for early diagnosis of AD (14).

In recent studies, measures of progressive AD-related atrophy detected from serial MRI scans show promise as biomarkers in evaluating the effectiveness of disease-modifying agents. So far, these studies have focused on relatively global measures, such as whole-brain and ventricular volume change (15, 16), although some have also looked at hippocampal volume change (17, 18). In these studies, despite the known regional specificity of AD-related volumetric changes, global measures have shown greater sensitivity than

local measurements, possibly because of the difficulty in obtaining accurate measurement of local brain structure change using existing methods (17). Nevertheless, these global measures of brain structure change are highly correlated with gold-standard clinical outcome measures, such as the Clinical Dementia Rating Scale Sum of Boxes and Mini Mental State Examination scores (15, 19).

The use of longitudinal anatomical quantification in multicenter clinical trials presents a number of challenges, including differences in MRI pulse sequences across scanner manufacturers, scanner-specific spatial distortions, and changes in scanner hardware and software over time that can affect image properties. In view of this, the Alzheimer's Disease Neuroimaging Initiative (ADNI) was designed to validate and compare imaging and biofluid markers of disease progression in a realistic multicenter clinical trial setting (20). The large, publicly available ADNI database thus provides a realistic setting in which to validate imaging methods aimed at assessing AD pathology. To this database, we applied a recently developed method for obtaining precise measures of interval change in cortical and subcortical regions, based on structural MRI, and determined the relative statistical power to discriminate pathology afforded by different regional measures.

## Results

We examined two models of treatable effects for power calculations. The first, Model T (for “total”), assumes that the study drug modifies both disease- and aging-related changes; the second, Model D (for “disease-specific”), assumes that the study drug modifies AD- or mild cognitive impairment- (MCI) related changes but has no effect on aging-related changes. We found that multiple regional volume changes, including those of whole brain, ventricle, hippocampus, entorhinal, fusiform, inferior temporal and middle temporal cortices, provided powerful outcome measures, with several measures requiring fewer than 100 subjects per arm to detect a 25% reduction in the rate of total change in AD, with 80% power at the  $P < 0.05$  significance level (see *Methods* for a description of the power calculations). Power calculations using ventricle and whole-brain volume change as outcome measures were particularly sensitive to the choice of treatable-effect model, especially in the case of MCI, where

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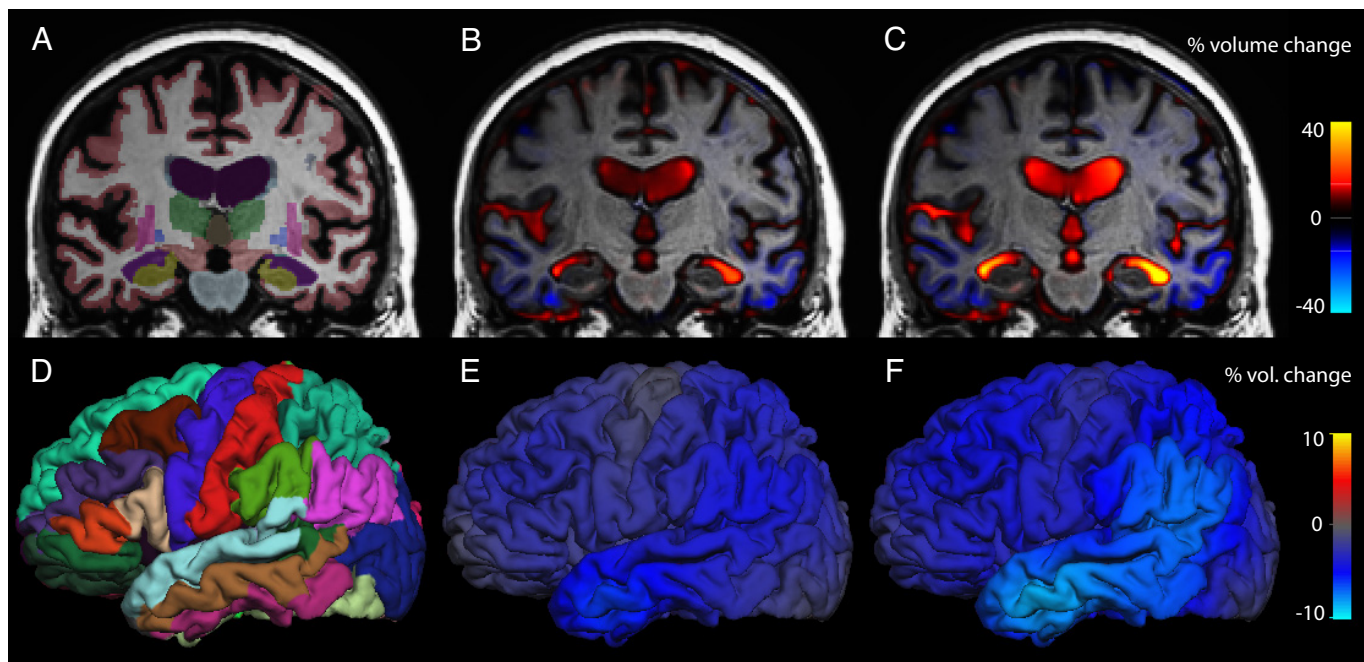
Conflict of interest statement: A.M.D. is a founder and holds equity in CorTechs Labs, Inc, and also serves on the Scientific Advisory Board. The terms of this arrangement have been reviewed and approved by the University of California at San Diego in accordance with its conflict of interest policies.

This article is a PNAS Direct Submission.

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<sup>2</sup>Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database ([www.loni.ucla.edu/ADNI](http://www.loni.ucla.edu/ADNI)). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators is available at [www.loni.ucla.edu/ADNI/Data/ADNIAuthorship.List.pdf](http://www.loni.ucla.edu/ADNI/Data/ADNIAuthorship.List.pdf).

This article contains supporting information online at [www.pnas.org/cgi/content/full/0906053106/DCSupplemental](http://www.pnas.org/cgi/content/full/0906053106/DCSupplemental).



**Fig. 1.** Tissue segmentation, with 6- and 12-month volume change fields for an MCI subject. (A) Segmentation of the baseline MRI scan, with different brain structures represented in different colors. (B) Corresponding coronal slice overlain with a heat map representation of the voxelwise estimates of volumetric change at 6 months and (C) 12 months. (D) Left hemisphere cortical parcellation of the baseline MRI scan. (E) Cortical surface overlain with a heat map representation of the estimates of cortical volumetric change at 6 months and (F) 12 months. Region-specific estimates were obtained by averaging the voxelwise change within each region of interest. In this subject, the left middle-temporal gyrus has decreased in volume by 4.7% at 6 months and by 8.2% at 12 months; the left temporal-horn lateral ventricle has increased by 17.4% at 6 months and by 35.3% at 12 months.

Model D required as much as six times the number of subjects per arm as Model T. When Model D was used for MCI, the best cognitive measure was as good as or outperformed these measures of more global structural change in the brain. For AD, regional cortical-volume change provided consistently superior power compared to cognitive measures regardless of choice of treatable effects model. The results indicate that volume loss in entorhinal, fusiform, inferior temporal and middle temporal cortices would serve as superior outcome measures for study drugs specifically targeting AD pathology in patients with MCI or AD.

Estimated changes across the brain at 6 and 12 months, along with cortical and subcortical tissue segmentation, are shown in Fig. 1 for an individual from the MCI cohort. Fig. 2 shows the results of power calculations for imaging measures of regional change, along with the best clinical cognitive-outcome measure, based on AD subjects and healthy controls. Results for Model T are in blue, and results for Model D are in red; numerical values are in Table 1 (see Fig. S1 and Table S1 for sample size estimates not incorporating random rates of change).

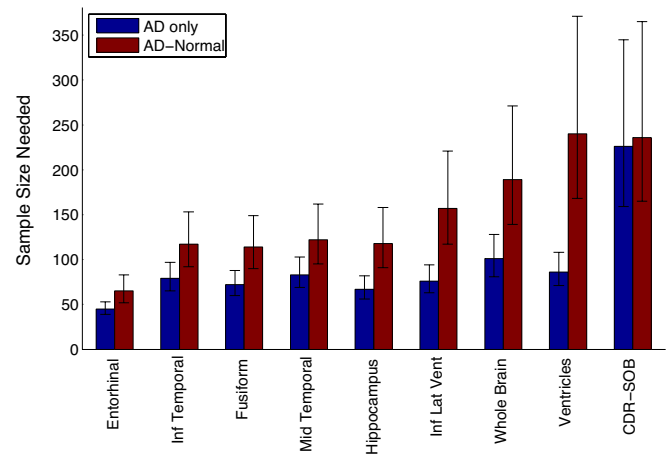
Imaging measures generally outperformed the best cognitive measure, regardless of model choice. While power estimates for cognitive measures were relatively unaffected by model choice, the power estimates for the imaging measures were strongly dependent on the treatment model used. Subregional cortical measures outperformed global imaging measures and were less dependent on choice of treatment model.

For MCI, as shown in Fig. 3 and Table 2, the dependence on model choice is even more salient than for AD. Notably, for ventricular volume, the sample size calculated using Model D is six times higher than that calculated using Model T, and exceeds that calculated for the best clinical or cognitive measure. Similar to what was found in AD, the regional temporal lobe cortical measures afforded the smallest sample sizes, regardless of model choice (see

Fig. S2 and Table S2 for sample size estimates not incorporating random rates of change).

## Discussion

The findings demonstrate that longitudinal volumetric change provides powerful outcome measures with which to examine putative disease-modifying medications for AD and MCI. Whole brain, ventricle, hippocampus, and cortical volumes of the entorhinal, fusiform, inferior temporal and middle temporal gyri undergo high rates of change in AD and MCI, which are



**Fig. 2.** Sample size estimates for AD from a linear mixed-effects model with random slopes. The bars, with 95% confidence intervals, indicate the expected number of subjects needed per arm to detect a 25% reduction in rate of change at the  $P < 0.05$  level with 80% power, assuming a 24-month trial with scans every 6 months. Results for Model T are in blue and results for Model D are in red; numerical values are shown in Table 1.

**Table 1. Sample size estimates (N) and annualized percent change for AD**

Measure	AD only N	AD-HC N	AD % change*	HC % change*
Entorhinal	45 [39 53]	65 [52 83]	-3.81 [-4.10 -3.52]	-0.64 [-0.85 -0.43]
Inf temporal	79 [65 97]	117 [92 153]	-3.64 [-4.00 -3.28]	-0.65 [-0.76 -0.53]
Fusiform	72 [60 88]	114 [90 149]	-2.90 [-3.17 -2.62]	-0.59 [-0.68 -0.50]
Mid temporal	83 [69 103]	122 [95 162]	-3.44 [-3.79 -3.09]	-0.60 [-0.73 -0.47]
Hippocampus	67 [56 82]	118 [91 158]	-3.28 [-3.58 -2.98]	-0.80 [-0.95 -0.65]
Inf lat vent	76 [63 94]	157 [117 221]	15.56 [14.04 17.09]	4.71 [3.95 5.47]
Whole brain	101 [81 128]	189 [139 271]	-1.50 [-1.67 -1.33]	-0.40 [-0.47 -0.34]
Ventricles	86 [71 108]	240 [168 371]	11.06 [9.91 12.21]	4.43 [3.83 5.03]
CDR-SOB	226 [159 345]	236 [165 365]	1.76 [1.43 2.10]	0.04 [0.00 0.07]
ADAS-Cog†	324 [217 536]	283 [192 457]	4.84 [3.76 5.92]	-0.34 [-0.59 -0.09]
MMSE†	482 [299 907]	494 [303 948]	-2.45 [-3.11 -1.78]	-0.03 [-0.14 0.08]

Values in brackets are 95% confidence intervals. ADAS-Cog, Alzheimer's Disease Assessment Scale—Cognitive; CDR-SOB, clinical dementia rating, sum of boxes; HC, healthy controls; MMSE, Mini-Mental State Examination.

\*Annual percent change in volume for all entries except CDR-SOB, ADAS-Cog, and MMSE.

†Not shown in Fig. 2.

quantifiable using serial MRI and the nonlinear registration procedures used here. A comparison of the current method with a standard method for quantifying global change is provided in the SI, where the analysis was restricted to a common data set of serial scans at 0, 6, and 12 months (Figs. S3 and S4 and Tables S3 and S4).

For clinical trial power calculations using longitudinal volumetric change as an outcome measure, choice of treatable-effect model influences which brain regions would be most sensitive to detect a drug effect, especially in MCI. If the drug is presumed to slow both age- and AD-related brain atrophy, then global and subregional medial temporal lobe (MTL) and cortical measures provide excellent statistical power to detect treatment effects. However, if the study drug is presumed to specifically slow AD-related brain atrophy, then subregional cortical measures provide superior power. For MCI, entorhinal cortex provided the most powerful outcome measure, which is consistent with findings suggesting that atrophy in this region is a sensitive marker of prodromal AD (11, 21).

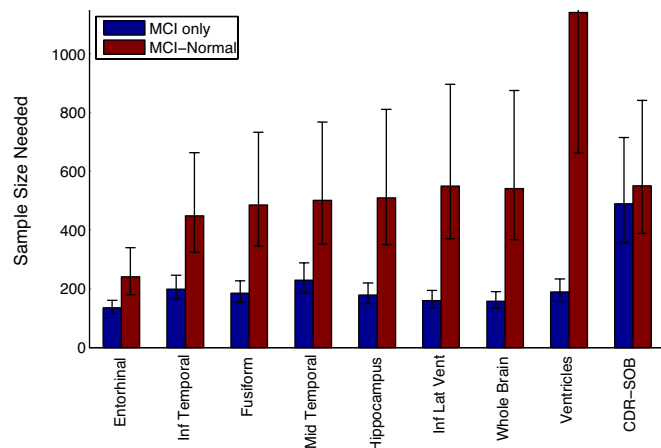
Choice of treatment model differentially affects cognitive and MRI variables; cognitive measures often show improvements over time in healthy controls because of practice effects (22), but deterioration over time in patients. Therefore, for cognitive measures, Model T can provide more conservative power estimates and is the most commonly used model in powering clinical trials. In contrast, both normal aging and disease are associated with atro-

phic changes over time. Thus, Model D generally provides more conservative power estimates for imaging measures. For this reason, it is important to consider both models when comparing across cognitive and imaging measures.

One of the primary motivations for using brain volumetric changes as outcome measures in clinical trials has been the evidence for greater statistical power afforded by such measures relative to clinical and cognitive measures (23). The present results, however, demonstrate that the most commonly used global imaging measures may be less powerful than the best clinical and cognitive measures, when the more conservative, and perhaps more realistic, disease-specific model is used. These effects are magnified in the MCI cohort, which is a patient population of particular interest for drug development (24, 25). Because of the overlap in behavioral features between MCI and healthy elderly controls, MCI trials would require particularly large subject numbers when using behavioral outcome measures alone.

Another motivation for using regional volumetric changes as outcome measures in clinical trials is the desire to more directly examine the effects of therapy on the brain's AD pathology. Because AD pathology is known to be concentrated in particular cortical and subcortical gray-matter regions, it would be desirable to measure change in the specific regions where neuronal dystrophy leads to pronounced atrophy. By itself, the halting of such neuronal dystrophy would lead to a stabilization of volume loss, but other drug effects, perhaps unrelated to therapeutic effect, may also be at play. For example, a recent active immunization trial against amyloid showed greater overall brain-volume loss in subjects who generated an immune response when compared to those who did not. In this case, global volume loss was attributed to possible changes in brain hydration state related to therapy. A trial of passive immunization against amyloid showed an association between higher doses of the medication and vasogenic edema. Thus, a short-term effect of the drug might be an increase in global brain volume that could be mistaken for a neuroprotection. Further study is needed to determine whether these processes are even more salient in regions enriched for amyloid and also whether such processes eventually reach a steady state upon which a drug's neuroprotective effect may still be evaluated. Nevertheless, regional measures of volumetric change offer a finer-grained examination of these processes and the effects of a therapy on the brain, and might be proportionally less affected by global effects unrelated to regional AD pathology (17, 26).

Although not a direct measure of the molecular pathology in AD, subregional brain structural changes are a direct measure of the neurodegeneration associated with the disease, and are more directly associated with progression of clinical symptoms than are measures of amyloid (27). Imaging of amyloid protein provides a



**Fig. 3.** Sample size estimates for MCI (see Fig. 2 for description). Numerical values are in Table 2. Note that the Model D (red) upper bound on the 95% confidence interval for ventricles is 2,421.

**Table 2. Sample size estimates (N) and annualized percent change for MCI**

Measure	MCI only N	MCI-HC N	MCI % change*	HC† % change*
Entorhinal	135 [115 161]	241 [180 340]	−2.54 [−2.75 −2.33]	−0.64 [−0.85 −0.43]
Inf temporal	199 [164 246]	449 [324 664]	−1.93 [−2.13 −1.74]	−0.65 [−0.76 −0.53]
Fusiform	185 [153 227]	485 [345 733]	−1.54 [−1.69 −1.39]	−0.59 [−0.68 −0.50]
Mid temporal	229 [186 288]	501 [353 768]	−1.84 [−2.04 −1.64]	−0.60 [−0.73 −0.47]
Hippocampus	179 [149 220]	510 [350 811]	−1.96 [−2.15 −1.78]	−0.80 [−0.95 −0.65]
Inf lat vent	160 [135 194]	550 [371 897]	10.23 [9.30 11.16]	4.71 [3.95 5.47]
Whole brain	158 [133 190]	541 [367 875]	−0.88 [−0.96 −0.80]	−0.40 [−0.47 −0.34]
Ventricles	189 [157 233]	1,141 [662 2,421]	7.47 [6.73 8.21]	4.43 [3.83 5.03]
CDR-SOB	490 [356 715]	551 [388 842]	0.67 [0.55 0.78]	0.04 [0.00 0.07]
ADAS-Cog‡	1,232 [748 2,403]	804 [500 1,502]	1.44 [1.03 1.84]	−0.34 [−0.59 −0.09]
MMSE‡	1,214 [744 2,322]	1,304 [751 2,800]	−0.84 [−1.08 −0.61]	−0.03 [−0.14 0.08]

\*Annual percent change in volume for all entries except CDR-SOB, ADAS-Cog, and MMSE.

†Normal values reproduced from Table 1.

‡Not shown in Fig. 3.

direct measure of one of the components of AD, but the sensitivity and specificity of this measure as a biomarker of AD remains an open question. There is growing evidence that amyloid protein may be elevated in some subjects who remain cognitively normal during the period of follow-up. Functional imaging measures (28–30) also show great promise as biomarkers in AD clinical trials and may be sensitive to pathology, at even earlier stages of disease.

An essential characteristic of an AD therapeutic is that it results in clinical or cognitive improvement. This improvement may be achieved through symptomatic modification (31, 32) or, preferably, through disease modification (33–36). Assessment of disease modification therefore relies on detecting a slowing of clinical decline. Clinical decline in AD occurs slowly over years, and so detecting a halt to this decline would be aided through complementary and sensitive measures. Although cognitive outcome is central to assessing therapeutic efficacy, cognitive decline is in fact a secondary effect of neuronal damage from the disease, partially reflected in regional atrophy. MRI longitudinal measures of regional volumetric change provide a valuable complement to cognitive measures in that they are not influenced by temporary symptomatic improvements, and they provide an early index of the drug's ability to reach the target organ and have an effect on AD-related atrophy.

Finally, regional volumetric measures of change show promise for eventual use in clinical practice to assist risk stratification and differential diagnosis at the earliest stages of neurodegenerative disease. These measures may be particularly powerful when combined with baseline volumetry (14) and other diagnostics, such as cerebrospinal fluid biomarkers, nuclear medicine ligands, neuropsychological tests, and genetics. The present results suggest that change in MTL cortical regions, in particular the entorhinal cortex, would provide the most sensitive and specific volumetric imaging measures early in the disease. Changes in regions such as the hippocampus, ventricles, and whole brain provide sensitive indices of disease progression but are also seen in healthy aging adults, thus reducing their specificity for the detection of AD.

## Methods

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

ADNI is the result of efforts of many coinvestigators from a broad range of academic institutions and private corporations. Subjects have been recruited from over 50 sites across the United States and Canada. ADNI's goal was to recruit 800 adults, ages 55 to 90, to participate in the research: ≈200 cognitively normal individuals to be followed for 3 years, 400 people with MCI to be followed for 3 years, and 200 people with early AD to be followed for 2 years (see [www.adni-info.org](http://www.adni-info.org)). The research protocol was approved by each local institutional review board and written informed consent is obtained from each participant.

**Participants.** The ADNI general eligibility criteria are described in the ADNI Protocol Summary page of the ADNI-Info Web site at [adni-info.org](http://adni-info.org) for 2009. Briefly, subjects are not depressed, have a modified Hachinski score of 4 or less, and have a study partner able to provide an independent evaluation of functioning. Healthy control subjects have a Clinical Dementia Rating (37) of 0. Subjects with AD have a Clinical Dementia Rating of 0.5 or 1.0 and meet National Institute of Neurological Disorders and Stroke and Alzheimer's Disease and Related Disorders Association criteria for probable AD (38).

In this study, we used baseline and follow-up data collected before August 27, 2009 from the ADNI database. Group clinical and demographic baseline data for the 169 healthy control, 299 MCI subjects, and 129 AD subjects in this study are presented in Table 3.

**Data Acquisition and Preparation.** Raw Digital Imaging and Communications in Medicine MRI scans, including two three-dimensional T1-weighted volumes per subject per visit, were downloaded from the public ADNI site ([www.loni.ucla.edu/ADNI/Data/index.shtml](http://www.loni.ucla.edu/ADNI/Data/index.shtml)). These data were collected across a variety of scanners with protocols individualized for each scanner, as defined at [www.loni.ucla.edu/ADNI/Research/Cores/index.shtml](http://www.loni.ucla.edu/ADNI/Research/Cores/index.shtml). In our laboratory, MRI data were reviewed for quality and automatically corrected for spatial distortion caused by gradient nonlinearity (39). For each subject at each visit, the two three-dimensional T1-weighted images were rigid-body aligned to each other, averaged to improve signal-to-noise ratio, and resampled to isotropic 1-mm voxels. Baseline volumetric segmentation (40, 41) and cortical surface reconstruction (42–45) and parcellation (46, 47) were performed using a data

**Table 3. Group demographics at baseline**

Group	HC subjects (n = 169)	MCI subjects (n = 299)	AD subjects (n = 129)
Age* (years)	76.2 ± 5.2	74.6 ± 7.4	74.6 ± 7.8
Female†	83 (49.1%)	111 (37.1%)	63 (48.8%)
Years of Education	16.0 ± 2.8	15.8 ± 3.0	15.0 ± 3.0
CDR-SOB	0.03 ± 0.12	1.56 ± 0.88	4.23 ± 1.54
ADAS-Cog	6.0 ± 2.8	11.6 ± 4.3	18.5 ± 6.2
MMSE	29.1 ± 1.1	27.0 ± 1.8	23.4 ± 2.0
APOE high risk	47 (27.8%)	170 (56.8%)	90 (69.8%)

APOE is apolipoprotein E gene; APOE risk was defined as the presence of the APOE e4 allele.

\*Data are mean ± standard deviation

†Data are numbers of subjects, and numbers in parentheses are percentages.

analysis pipeline based on the FreeSurfer software package and customized Matlab code, optimized for use on large multisite data sets. The automated whole-brain segmentation procedure uses a probabilistic atlas and applies a Bayesian classification rule to assign a neuroanatomic label to each voxel. The atlas consists of a manually derived training set created by the Center for Morphometric Analysis (Massachusetts General Hospital, Harvard Medical School) from 40 non-ADNI subjects across the adult age range, including individuals with AD. Automated volumetric segmentation required only qualitative review to ensure that there was no technical failure of the application.

The cortical surface was reconstructed to measure thickness at each surface location, or vertex, to allow visualization of group differences at each vertex. The surface was parceled into distinct regions of interest (ROIs). The cortical-surface model was manually reviewed and edited for accuracy. Minimal editing was performed according to standard, objective rules, including correction of errors in removal of nonbrain areas and inclusion of white-matter areas of hypointensity adjacent to the cortical ribbon. Qualitative review and editing were performed, with blinding to the diagnostic status, by one of three technicians trained and supervised by an expert neuroanatomist with more than 10 years of experience (C.F.-N.). The technicians had a minimum of 4 months of experience reviewing brain MR images before their involvement in this project.

Qualitative review and editing required  $\approx 45$  min per subject. Baseline image construction was carried out on a Linux cluster composed of dual quad-core 2.5 GHz CPUs (Xeon E5420; Intel) with 16 GB RAM; each image reconstruction was run as an independent process and took  $\approx 24$  h of computational time.

**Estimation of ROI Volumetric Interval Change.** For each subject, follow-up images were fully affine-registered to the baseline image, and their intensities brought into local agreement (i.e., corrected for relative  $B_1$ -induced intensity distortion). Nonlinear registration of the images was then performed, where voxel centers are moved about until a good match between the images is made. Several methods exist for causing this to happen, including fluid deformation (48–50) and tensor-based morphometry (51). For the results presented here, however, we developed and applied a method (52) based on linear elasticity and closer in spirit to tensor-based morphometry. This method proceeds essentially as follows. The images are heavily blurred (smoothed), making them almost identical, and a merit or potential function calculated. This merit function expresses the intensity difference between the images at each voxel, and depends on the displacement field for the voxel centers of the image being transformed; it is also regularized to keep the displacement field spatially smooth. The merit function by design will have a minimum when the displacement field induces a good match between the images. The displacement field in general will turn cubic voxels into displaced, irregular hexahedra whose volumes (53) give the volume-change field. The merit function is minimized efficiently using standard numerical methods. Having found a displacement field for the heavily blurred pair of images, the blurring is reduced and the procedure repeated, thus iteratively building up a better displacement field. Two important additions to this are: (i) applying the final displacement field to the image being transformed, then nonlinearly registering the resultant image to the same target, and finally tracing back through the displacement fields, thus calculated to find the net displacement field; and (ii) restricting to ROIs and zooming when tissue structures are separated by only a voxel or two. These additional features enable very precise registration involving large or subtle deformations, even at small spatial scales with low boundary contrast.

All available healthy controls, MCI subjects, and subjects with AD who passed the qualitative baseline review described above were thus registered. From the deformation field, a volume-change field was calculated; an example is shown in Fig. 1. For each subject, the volume-change field was averaged over each ROI, including those of the cortical surface (change in cortical volume to first-order results from change in thickness), to give the percentage change from baseline. Further visual quality control, blind to diagnosis, was carried out by a technician on the volume-change field to exclude cases where there was significant degradation in meaningful registration for at least one ROI because of artifacts or major changes in scanner hardware between visits (e.g., change of scanner model or type of RF coil). The most common form of artifact, affecting approximately half of the rejected scans, was caused by within-scan subject motion. In future clinical trials, the loss of scans caused by motion artifacts may be greatly reduced

by using real-time motion-correction procedures (54, 55). Artifacts resulting from change in scanner models between visits typically include differential contrast or spatial blurring, mostly affecting the fine-scale estimates of change (e.g., within the cortical ROIs). Artifacts resulting from change in RF coil, specifically from a traditional quadrature head coil to a phased-array coil, primarily resulted in dramatic changes in blood inflow effects, which in turn predominantly affected MTL measures. The combination of artifacts affecting the volume change field reduced the number of healthy control follow-up scans by 14.2%, the number of MCI follow-up scans by 14.5%, and the number of AD follow-up scans by 15.8%.

For a subject to be included in our statistical analyses, several criteria needed to be satisfied: the baseline cortical parcellation and subcortical segmentation had to pass review, as described above; for a tight comparison between cognitive and volumetric measures, a subject's follow-up was eliminated unless both volumetric and cognitive data, including a clinical diagnosis, existed for that follow-up; there was at least one good follow-up, along with the good baseline; a healthy control needed to remain such at all follow-ups; and finally, the volume-change field had to pass review. Quality control on the volume-change field reduced the number of healthy controls by 8.6% to 169, the number of MCI subjects by 8.5% to 299, and the number of AD subjects by 12.2% to 129.

**Power Calculations.** We examined two models of treatable effects for power calculations: Model T assumes that the study drug modifies both disease- and aging-related changes; Model D assumes that the study drug modifies only AD- or MCI-related changes.

Power calculations were performed using a mixed-effects regression model for the outcome variable (absolute cognitive measure or subregional percent-volume change) as a linear function of time, with random (individual-specific) slope or trend term and, for the cognitive measures, random intercept (baseline value). Sample sizes per arm were estimated based on a z-test (56) for absolute mean slopes for AD and MCI subjects (Model T), and the difference in mean slopes for AD and MCI subjects from healthy controls (Model D). The sample size required to detect 25% slowing in mean rate of decline for a hypothetical disease-modifying treatment versus placebo was estimated for a 24-month, two-arm, equal-allocation trial, with a 6-month assessment interval. Power calculations were performed with the requirement that the trial have 80% power to detect the treatment effect using a two-sided significance level of 5%. The sample size per arm scales with the variance of the within-group rate of change (slope), which has both between-subject and within-subject (residual error variance of the mixed-effects model) components. Thus, for Model T, the treatment-effect size of interest was 25% of the rate of change in the patient population (MCI or AD), and for Model D it was 25% of the difference between the rates of change in the patient and normal populations. Confidence intervals of 95% for sample sizes were based on 95% confidence intervals for the treatment-effect size of interest. Power calculations were implemented in Matlab version 2008b, using the nlme function in the Statistics Toolbox. Sample size estimates based on a linear random-effects model ignoring between-subject variance in the rate of change (i.e., taking the group-specific rate of change as a fixed effect) are provided in Figs. S1 and S2, and Tables S1 and S2.

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