Alzheimer's & Dementia 10 (2014) 511–521

Featured Articles

Biological heterogeneity in ADNI amnestic mild cognitive impairment

Jasmine Nettiksimmons[a,*], Charles DeCarli[b], Susan Landau[c], Laurel Beckett[d], for the Alzheimer's Disease Neuroimaging Initiative

[a]*Department of Psychiatry, University of California–San Francisco, San Francisco, CA, USA*
[b]*Department of Neurology and Center for Neuroscience, University of California–Davis, Sacramento, CA, USA*
[c]*Helen Wills Neuroscience Institute, University of California–Berkeley, Berkeley, CA, USA*
[d]*Division of Biostatistics, Department of Public Health Sciences, University of California–Davis, Davis, CA, USA*

Abstract

Background: Previous work examining normal controls from the Alzheimer's Disease Neuroimaging Initiative (ADNI) identified substantial biological heterogeneity. We hypothesized that ADNI mild cognitive impairment (MCI) subjects would also exhibit heterogeneity with possible clinical implications.

Methods: ADNI subjects diagnosed with amnestic MCI ($n = 138$) were clustered based on baseline magnetic resonance imaging, cerebrospinal fluid, and serum biomarkers. The clusters were compared with respect to longitudinal atrophy, cognitive trajectory, and time to conversion.

Results: Four clusters emerged with distinct biomarker patterns: The first cluster was biologically similar to normal controls and rarely converted to Alzheimer's disease (AD) during follow-up. The second cluster had characteristics of early Alzheimer's pathology. The third cluster showed the most severe atrophy but barely abnormal tau levels and a substantial proportion converted to clinical AD. The fourth cluster appeared to be pre-AD and nearly all converted to AD.

Conclusions: Subjects with MCI who were clinically similar showed substantial heterogeneity in biomarkers.

Keywords: ADNI; Alzheimer's disease; Clustering; Heterogeneity; Amnestic MCI

1. Introduction

The Alzheimer's Disease Neuroimaging Initiative (ADNI) was designed to have three clinically distinct study groups: cognitively normal controls, individuals with mild cognitive impairment (MCI) who had a memory complaint but did not have significant impairment in other domains, and a group of individuals with mild Alzheimer's disease (AD) who met National Institute of Neurological and Communicative Disorders and Stroke (NINCDS)/Alzheimer's Disease and Related Disorders Association (ADRDA) criteria for probable AD. These groups were designed to be reasonably homogeneous within their respective diagnostic categories. Despite attempts to acquire clinically homogeneous groups at baseline, we previously found that normal controls had considerable underlying biological heterogeneity that was associated with cognitive differences [1]. We hypothesized that the MCI group also contains substantial biological heterogeneity, also with possible clinical consequences.

Amnestic MCI is a subset of MCI that is often thought of as prodromal-AD. A substantial proportion of individuals with amnestic MCI will ultimately convert to AD over 2 to 5 years [2,3]. The current amyloid cascade hypothesis of AD suggests that the process begins with amyloid-β (Aβ) plaque deposition, followed by measurable changes in cerebrospinal fluid (CSF) tau proteins, then changes in brain volume, and finally clinically detectable cognitive change [4]. However, not all individuals with MCI progress to dementia, and there is heterogeneity in the underlying pathology of those that do progress [5]. In an autopsy study, Jicha and colleagues found that a substantial number of amnestic subjects had primary pathologies other than AD [3].

*Corresponding author. Tel.: +415-221-4810 ext. 6565. Fax: +415-379-5624.
E-mail address: jasmine.nettiksimmons@ucsf.edu

1552-5260/$ - see front matter
http://dx.doi.org/10.1016/j.jalz.2013.09.003

Possible non-AD pathologies that may produce amnestic MCI include vascular dementia, hippocampal sclerosis, and frontotemporal dementia (FTD) [6–9]. It is also common for multiple cognitive pathologies to coexist, which adds to the difficulty of clinical and pathological classification [10].

Understanding the biological and clinical heterogeneity represented by the seemingly specific clinical diagnosis of amnestic MCI is essential to understanding the pathways involved in cognitive decline and ultimately necessary for the development of treatment. The goal of this analysis was to characterize the heterogeneity in the ADNI amnestic MCI group. Cluster analysis allows an unbiased characterization of the data—free from artificial cutoffs or dichotomizations that remove meaningful variability. Subjects in this study were clustered to see if common patterns exist in the biomarker profiles of the MCI participants, and these profiles were compared with the anticipated pattern of biomarkers for prodromal AD prescribed by the amyloid cascade hypothesis [4]. Next, clusters were characterized and tested for association with subject characteristics and clinical outcomes.

2. Methods

2.1. Subjects

The data used for this analysis were downloaded from the ADNI database (www.loni.ucla.edu/ADNI) on December 14, 2011. The individuals studied were recruited between August 17, 2005 and September 4, 2007 as ADNI participants. Additional details are given in Petersen et al. [11]. This study was approved by the Institutional Review Boards of all participating institutions. Informed written consent was obtained from all participants at each site. A detailed description of the study design and inclusion criteria is available at clinicaltrials.gov/show/NCT00106899.

All enrolled ADNI MCI subjects were diagnosed as having amnestic MCI; this diagnostic classification required Mini-Mental State Examination MMSE scores between 24 and 30 (inclusive), a memory complaint, objective memory loss measured by education-adjusted scores on the Wechsler Memory Scale Logical Memory II, a Clinical Dementia Rating (CDR) of 0.5, absence of significant impairment in other cognitive domains, essentially preserved activities of daily living, and absence of dementia. The ADNI MCI diagnostic group contained 382 individuals. Of those, 189 individuals consented to CSF testing. Baseline magnetic resonance imaging (MRI) scans failed to meet quality control in 18 subjects, 32 subjects had missing values for white matter hyperintensity (WMH), and two subjects were missing homocysteine data. One hundred thirty-eight MCI subjects had complete baseline data for the variables chosen for this analysis.

2.2. Measures

The biological focus of the analysis was on 11 variables: total brain volume, hippocampal volume, ventricle volume, entorhinal cortex thickness, CSF Aβ ($A\beta_{1\text{-}42}$), CSF total tau (tau), CSF phosphorylated tau (P-$tau_{181\ P}$), the ratio of tau to $A\beta_{1\text{-}42}$, the ratio of P-$tau_{181\ P}$ to $A\beta_{1\text{-}42}$, WMH, and serum homocysteine. All MRI summary volumes were calculated as fractions of the total intracranial volume, which included the area occupied by the brainstem inside of the skull, and were calculated using Quarc, a modification of Free-Surfer implemented by the Anders Dale Laboratory at the University of California–San Diego as part of the ADNI shared dataset [12,13]. Individual longitudinal changes were calculated from cross-sectional Quarc summaries at available follow-up times by fitting mixed models [14]. WMHs were detected by the Imaging of Dementia and Aging (IDeA) laboratory at the University of California–Davis based on coregistered T1-, T2-, and proton density (PD)-weighted images using an automated protocol [15]. CSF samples were batch processed by the ADNI Biomarker Core at the University of Pennsylvania School of Medicine [16]. This set of biomarkers was chosen to view the biology underlying cognitive impairment from multiple perspectives by including measures of abnormal protein activity associated with AD, region-specific atrophy, vascular damage, and a blood serum measure associated with dementia. The clinical outcomes used in this analysis were the following cognitive tests: Digit Span forward and backward, Digit Symbol Substitution, Trails B, Category Naming (sum of animal and vegetable scores), the MMSE, Alzheimer's Disease Assessment Scale cognitive subscale, the sum of five trials from the Rey's Auditory Vocabulary List Test (RAVLT), and Logical Memory II [17–23].

2.3. Statistical analysis

Cluster analysis provides a unique opportunity to group individuals on the basis of their biomarkers that is not based on (or biased by) artificial cutoffs or long-term trajectories. The goal of cluster analysis is to separate individuals into groups such that individuals within a group are as similar to each other as possible and groups are as different from each other as possible. To cluster the MCI subjects, we used agglomerative hierarchical clustering with Ward's method of minimum variance and the Euclidean distance metric. This method seeks to minimize the variance of the distances from each individual in a cluster to the cluster center, thereby ensuring similarity of the individuals within a cluster. In preparation for clustering, each clustering variable was standardized by the overall MCI mean and standard deviation (SD) for that variable so that variables on different numerical scales could be fairly compared. The number of clusters was not determined a priori. Instead, the number was chosen using the maximum gap statistic, but in a less conservative manner than originally described, which showed promising results in simulations [24], along with visual ascertainment of cluster separation and minimum sample size considerations.

Analysis of variance (ANOVA) F statistics and exact tests were used to test for baseline differences in

demographics, genetics, and baseline cognitive scores among the four clusters. Comparisons of cognitive test scores at baseline did not control for education or age. Baseline biomarkers are reported with 95% confidence intervals (CIs) because significant differences between the clusters were to be expected. Linear regression models with random slopes and intercepts were used to estimate adjusted baseline measures and test for longitudinal differences in anatomical and cognitive change. Models of anatomical and cognitive outcomes controlled for age (centered) and the interaction of age with time. Models of cognitive outcomes also controlled for years of education (centered). Time to conversion was assessed with an accelerated failure time (AFT) model assuming a Weibull distribution and controlling for age. The interval-censored AFT model was parameterized in two different ways. First, the healthiest looking cluster (MCI 1) was made the reference and all others were compared to it. Next, the model was parameterized in the following way to achieve sequential comparisons: MCI 2:MCI 1, MCI 3:MCI 2, and MCI 4:MCI 3. Individuals who were classified as MCI at baseline were included in these analyses, even if they later were reported as normal. *P* values for cluster comparisons were not adjusted for multiple comparisons. All analyses were performed in R 2.10.0 and R 2.11.0 [25].

3. Results

3.1. Baseline differences

Four clusters were found; Figure 1 displays some of the biomarker characteristics in what are essentially small bin histograms showing each subject (means from the normal controls and AD subjects in ADNI are provided as reference; more information on the ADNI control and AD subjects can be found in Supplemental Table 1). The clusters can be biologically summarized as follows. MCI 1 ($n = 20$) had the healthiest biomarker profile, which was often centered around the normal control mean and was

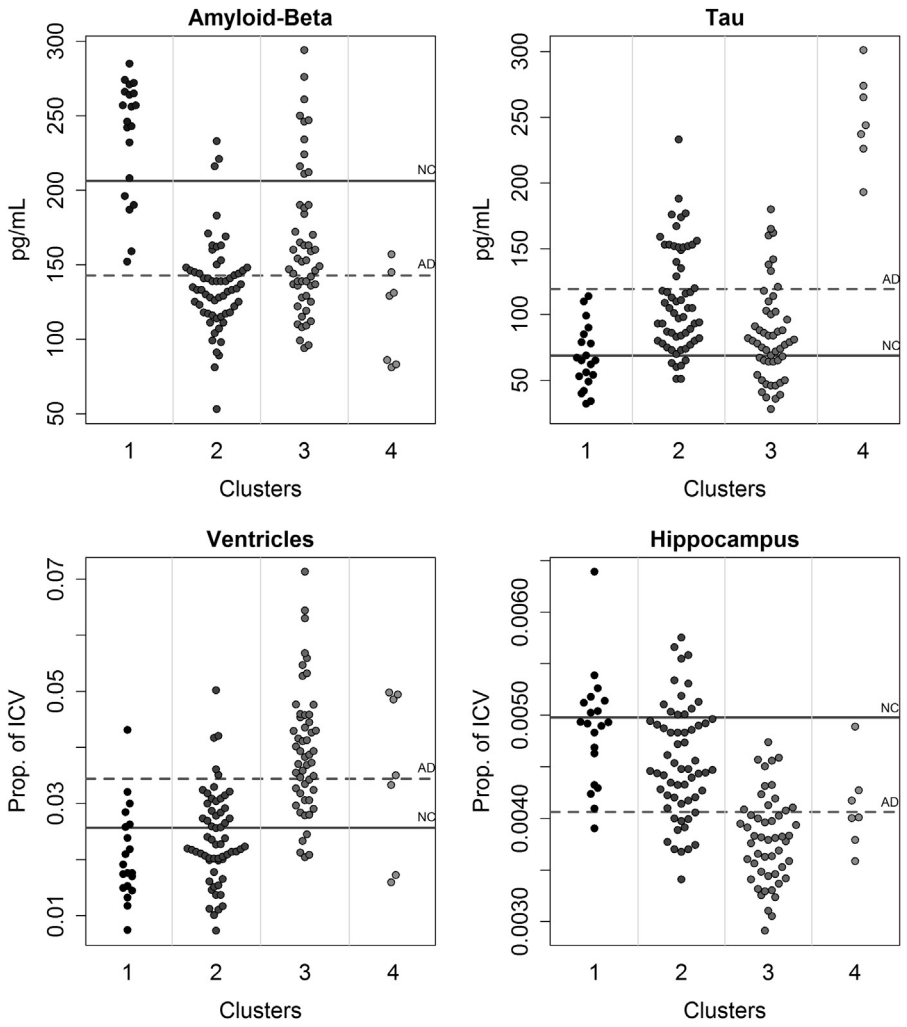

Fig. 1. Distributions by cluster for a subset of clustering biomarkers. The solid gray line represents the mean from the ADNI normal controls and the dashed line represents the AD mean. ICV, intracranial volume; ADNI, Alzheimer's Disease Neuroimaging Initiative; AD, Alzheimer's disease.

sometimes less abnormal than the normal control mean (Figure 1). MCI 4 ($n = 7$) had the least healthy biomarker profile, which was often near or beyond the averages in the AD group. MCI 2 ($n = 60$) was between MCI 1 and MCI 2 and had characteristics suggestive of the early stages of AD, as described in the Jack et al. model [4]. MCI 3 ($n = 51$) was similar in many ways to MCI 2, but it had substantially lower tau and much worse MRI measures. The clusters differed on age (Table 1); post hoc testing using Tukey's honest significant difference method indicated that MCI 1 and 2 were significantly younger than MCI 3 and 4, but MCI 1 and 2 did not differ significantly from each other, and MCI 3 and 4 did not differ significantly from each other. Table 2 shows the means and 95% CIs in each cluster for all of the clustering biomarkers; here the extent of cluster separation on each biomarker can be seen. There were significant differences among the clusters on the Digit Symbol Substitution test and Trails B as well as all memory tests (Table 1). In general, MCI 1 had the best cognitive scores, MCI 2 and 3 were in the middle and often very close to one another, and MCI 4 had the most severe impairment. This cognitive continuum might suggest that all of the clusters are on the same path to AD, with some simply farther along the path. However, the plot of $A\beta_{1\text{-}42}$, tau, hippocampal volume, and ventricle volume in Figure 2 suggests that this is not the case. For this figure, group z scores have been scaled by the cognitively normal group's mean and SD; therefore, zero represents true cognitive normality, not the average of MCI subjects. If all of the individuals were on the same biological path, then one would expect that the clusters could be ordered with increasing abnormality. Instead, the plot demonstrates a lack of consistent ordering, with more abnormal CSF measures in MCI 2 than MCI 3.

3.2. Atrophy

The clusters were created using baseline MRI measurements and consequently showed significant differences in atrophy at baseline, but the clusters also had significantly different rates of atrophy over time (Figure 3). MCI 1 (reference group) showed atrophic change for brain (-0.004, $P < .001$), hippocampus (-6.58×10^{-5}, $P < .001$), entorhinal cortex (-4.44×10^{-8}, $P = .01$), and ventricular volume (0.001, $P = .04$). Compared with the reference group, MCI 2 had a significantly worse atrophic change in the entorhinal cortex (-5.66×10^{-8} additional decline per year, $P = .002$) and ventricular volume

Table 1
Cluster comparison on baseline demographic, genetic, clinical, and biomarker measures

	MCI 1 ($n = 20$)	MCI 2 ($n = 60$)	MCI 3 ($n = 51$)	MCI 4 ($n = 7$)	
Measures	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	*P*
Demographic					
Age (years)	71.7 ± 8	72.2 ± 7.2	76.7 ± 7.1	76.3 ± 3.6	**.001**
Gender (male)	60%	55%	73%	57%	.27
Education (years)	15.5 ± 2.7	15.9 ± 3.1	16.2 ± 3	15.7 ± 1.8	.50
APOE (∈4 carrier)	40%	60%	51%	71%	.44
Parent with AD	25%	38%	20%	29%	.34
Follow-up (years)	2.8 ± 0.5	2.7 ± 0.6	2.5 ± 0.6	2.6 ± 0.6	.08
Clinical					
FAQ	1.8 ± 3.2	4.3 ± 4.7	3.8 ± 4.4	3.1 ± 3.9	.38
Geriatric Depression	1.8 ± 1.1	1.5 ± 1.4	1.6 ± 1.1	1.1 ± 0.7	.56
Attention					
Digit Span forward	8 ± 2.2	8.2 ± 2.3	8.5 ± 2.1	8 ± 1.4	.50
Digit Span backward	7.2 ± 2	6.2 ± 1.9	6.1 ± 2	7.3 ± 1.3	.38
Executive					
Digit Symbol	45 ± 11.4	36.8 ± 11.6	34.6 ± 9.4	39.6 ± 9.4	**.01**
Trails B	85.9 ± 51.9	132 ± 76.7	141.4 ± 66.2	148.7 ± 61.3	**.01**
Category Naming	29.3 ± 6.1	26.9 ± 7.4	27.8 ± 8.1	21.7 ± 6.4	.18
Memory					
MMSE	27.6 ± 1.7	27 ± 1.8	26.4 ± 1.8	26.9 ± 2	**.02**
ADAS-cog	9.8 ± 4.4	11.8 ± 4.4	12.3 ± 4.8	12.8 ± 4.2	**.046**
RAVLT (sum of five trials)	34.5 ± 10.3	30.3 ± 8.3	28.5 ± 8.1	29.1 ± 6.9	**.02**
RAVLT 30 min	4.3 ± 3.6	2.8 ± 3.6	1.8 ± 2.2	0.9 ± 1.6	**.001**
RAVLT (% savings)	0.5 ± 0.3	0.3 ± 0.4	0.2 ± 0.2	0.1 ± 0.3	**.001**
Logical Memory II	5.3 ± 2.4	3.4 ± 2.8	3.5 ± 2.5	2.3 ± 2.1	**.01**

Abbreviations: MCI, mild cognitive impairment; SD, standard deviation; *APOE*, apolipoprotein E; AD, Alzheimer's disease; FAQ, Functional Activity Questionnaire; MMSE, Mini-Mental State Examination; ADAS-cog, Alzheimer's Disease Assessment Scale - cognitive subscale; RAVLT, Rey's Auditory Vocabulary List Test; ANOVA, analysis of variance.

NOTE. Means and SD are reported unless otherwise noted. Confidence intervals (95%, two-sided) have been reported for the clustering biomarkers because significant differences are expected. *P* values come from ANOVA F statistics and exact tests for categorical variables. Bold p-values indicate $p<0.05$.

*Proportion with at least one ε4 allele.

Table 2
Means and associated 95% confidence intervals for clustering biomarkers by cluster

Biomarkers	MCI 1	MCI 2	MCI 3	MCI 4
Whole brain volume*	0.6982 (0.6888, 0.7076)	0.6801 (0.6747, 0.6855)	0.6586 (0.6527, 0.6645)	0.659 (0.6431, 0.6748)
Hippocampal volume*	0.0049 (0.0046, 0.0051)	0.0046 (0.0044, 0.0047)	0.0038 (0.0037, 0.0039)	0.0041 (0.0037, 0.0045)
Ventricle volume*	0.0209 (0.0165, 0.0252)	0.0239 (0.0214, 0.0264)	0.0395 (0.0368, 0.0422)	0.0356 (0.0282, 0.0429)
Entorhinal thickness†	3.206 (3.0327, 3.3792)	3.0501 (2.95, 3.1501)	2.5764 (2.4679, 2.6849)	2.3871 (2.0943, 2.6800)
WMH‡	1.433 (0.558, 2.308)	2.557 (2.051, 3.062)	2.52 (1.972, 3.068)	3.624 (2.145, 5.103)
$A\beta_{1\text{-}42}$§	236.1 (218.5, 253.7)	135.3 (125.1, 145.5)	163.7 (152.7, 174.8)	116 (86.2, 145.8)
Tau§	67.2 (51.2, 83.1)	111.5 (102.3, 120.7)	84.5 (74.5, 94.4)	248.6 (221.7, 275.5)
P-tau_{181}§	21.1 (15, 27.1)	42.7 (39.3, 46.2)	29.8 (26, 33.6)	68.3 (58.1, 78.4)
P-tau/Aβ	0.3 (0.16, 0.44)	0.86 (0.78, 0.94)	0.57 (0.48, 0.66)	2.3 (2.06, 2.54)
Tau/Aβ	0.1 (0.04, 0.15)	0.33 (0.3, 0.37)	0.2 (0.17, 0.23)	0.62 (0.53, 0.7)
Homocysteine‖	10.31 (9.07, 11.56)	9.64 (8.92, 10.36)	10.81 (10.03, 11.59)	10.11 (8, 12.22)

Abbreviations: MCI, mild cognitive impairment; WMH, white matter hyperintensities; P-tau, phosphorylated tau; Aβ, amyloid-β.
*Presented as fraction of intracranial volume.
†Average of right and left in millimeters.
‡Cubic centimeters.
§Picograms per milliliter.
‖Micromoles per liter.

(0.001 additional increase per year, $P = .01$), but not for hippocampal or total brain volumes ($P > .15$). MCI 3 and 4 experienced significantly greater annual change than MCI 1 on every measure, with rates of change approximately 2 to 4 times greater than MCI 1 (all $P \leq .01$).

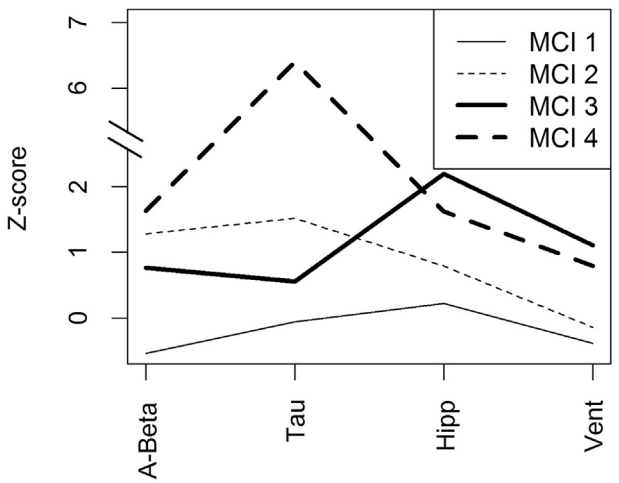

Fig. 2. The z scores shown were created by subtracting the ADNI normal mean and dividing by the ADNI normal standard deviation for each biomarker shown so that zero represents actual normality, not normality within the MCI subgroup. Signs have been reversed so that large, positive values always indicate more severe damage. A-Beta, amyloid-β; Hipp, hippocampal volume; Vent, ventricle volume; ADNI, Alzheimer's Disease Neuroimaging Initiative; MCI, mild cognitive impairment.

3.3. Cognitive decline

Groups also differed significantly in baseline and longitudinal cognitive performance (Table 3). Baseline performance differed on several memory and executive function tests, but not on the MMSE. Over time, the models showed significant differences between groups in the areas of memory and executive function. For example, MCI 1 generally showed no change in cognitive performance and even showed significant improvement on the Logical Memory II test (0.75 points per year, $P < .001$). In contrast, MCI 2 showed significant decline on every test except Digit Span (forward and backward) and Logical Memory II, although Logical Memory II was nearly significant (95% CI: −0.46, 0.06). MCI 3 and MCI 4 showed global declines on nearly all measures. For the few cognitive measures in which MCI 3 and 4 did not exhibit significant decline, the baseline values for MCI 3 and 4 were substantially worse, suggesting possible plateauing.

3.4. Conversion to AD

After an average of 2.6 years of follow-up, 70 subjects (51%) converted to AD: 4 conversions in MCI 1

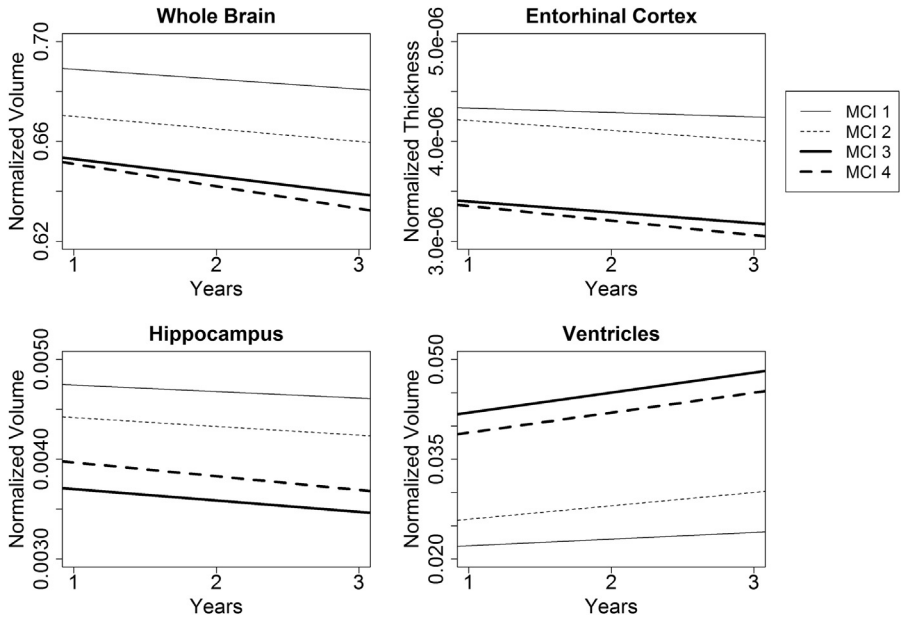

Fig. 3. Estimated atrophy in all four MCI clusters over a 3-year period. Estimates come from linear mixed-effects regression models with random effects for slope and intercept and control for age and the interaction of age with time. There were significant differences between MCI 1 and MCI 3 and 4 for all measures. MCI 1 differed from MCI 2 in ventricles and entorhinal cortex at baseline and brain and hippocampus atrophy over time. Secondary analysis excluding MCI 1 and using MCI 3 as the referent found no significant differences in intercept between MCI 3 and MCI 4 for any of the MRI measures. MCI 4 experienced significantly more rapid deterioration in the entorhinal cortex than MCI 3. MCI 2 and MCI 3 differed significantly in slope and intercept for all MRI measures. MCI, mild cognitive impairment; MRI, magnetic resonance imaging.

Table 3
Adjusted baseline and annual change results (coefficients, standard errors, and *P* values) from longitudinal linear regression models with random effects for slope and intercept

	MC1 (referent)		MCI 2 (vs MCI 1)		MCI 3 (vs MCI 1)		MCI 4 (vs MCI 1)	
Cognitive tests	Est ± SE	*P*	Est ± SE	*P*	Est ± SE	*P*	Est ± SE	*P*
Baseline								
Digit Span forward	8.28 ± 0.43	<.0001	−0.05 ± 0.49	.91	−0.13 ± 0.51	.80	−0.65 ± 0.83	.44
Digit Span backward	7.37 ± 0.41	<.0001	−1.31 ± 0.47	**.01**	−1.29 ± 0.49	**.01**	−0.72 ± 0.80	.37
Digit Symbol	46.50 ± 2.47	<.0001	−8.92 ± 2.8	**.002**	−12.10 ± 2.95	**.0001**	−5.12 ± 4.81	.29
Trails B	84.76 ± 15.21	<.0001	44.4 ± 17.3	**.01**	61.26 ± 18.18	**.001**	73.61 ± 29.58	**.01**
Category Naming	29.31 ± 1.53	<.0001	−2.42 ± 1.74	.17	−2.10 ± 1.83	.25	−5.73 ± 2.98	.06
ADAS-cog	9.22 ± 0.97	<.0001	2.07 ± 1.11	.06	3.05 ± 1.16	**.001**	2.74 ± 1.90	.15
RAVLT (sum of five)	34.00 ± 1.83	<.0001	−4.21 ± 2.08	**.045**	−6.29 ± 2.19	**.005**	−6.87 ± 3.57	.06
Logical Memory II	5.83 ± 0.72	<.0001	−2.21 ± 0.82	**.01**	−2.55 ± 0.86	**.004**	−3.83 ± 1.41	**.008**
MMSE	27.72 ± 0.63	<.0001	−1.04 ± 0.72	.15	−1.33 ± 0.76	.08	−0.79 ± 1.24	.52
Annual change								
Digit Span forward	0.03 ± 0.12	.79	−0.09 ± 0.14	.51	−0.44 ± 0.14	**.002**	−0.40 ± 0.24	.09
Digit Span backward	−0.24 ± 0.16	.13	0.10 ± 0.18	.56	−0.05 ± 0.19	.80	−0.50 ± 0.33	.13
Digit Symbol	0.53 ± 0.71	.45	−2.82 ± 0.81	**<.0001**	−2.75 ± 0.86	**.001**	−6.24 ± 1.44	**<.0001**
Trails B	3.97 ± 5.48	.47	10.88 ± 6.32	.09	8.01 ± 6.68	.23	3.21 ± 10.92	.77
Category Naming	−0.08 ± 0.58	.89	−1.32 ± 0.67	.05	−2.00 ± 0.71	**.005**	−3.61 ± 1.17	**.002**
ADAS-cog	−0.26 ± 0.59	.66	1.72 ± 0.68	**.01**	2.74 ± 0.71	**.0001**	6.33 ± 1.19	**<.0001**
RAVLT (sum of five)	−0.30 ± 0.46	.51	−0.80 ± 0.54	.14	−1.61 ± 0.56	**.005**	−4.10 ± 0.97	**<.0001**
Logical Memory II	0.75 ± 0.22	**<.0001**	−0.95 ± 0.26	**.0002**	−1.37 ± 0.27	**<.0001**	−1.56 ± 0.45	**<.0001**
MMSE	0.09 ± 0.21	.68	−0.78 ± 0.24	**.001**	−1.43 ± 0.25	**<.0001**	−2.56 ± 0.41	**<.0001**

Abbreviations: MCI, mild cognitive impairment; Est., estimate; SE, standard error; MMSE, Mini-Mental State Examination; ADAS-cog, Alzheimer's Disease Assessment Scale - cognitive subscale; RAVLT, Rey's Auditory Vocabulary List Test.

NOTE. Cluster was used as a predictor in models with cognitive scores as outcomes. All models were adjusted for age, age*time, and education. Age and education have been centered; results are for individuals of average age and education. The intercept is the average baseline level of MCI 1. The rest of the baseline values show how the other clusters differ from MCI 1. The reference slope is the slope for MCI 1. The rest of the annual-change values show the difference in slope between the rest of the clusters and MCI 1. The significant *P* values for the baseline referent are for the trivial test that the intercept equals zero. Bold p-values indicate P<.05.

(20%), 32 conversions in MCI 2 (53%), 28 conversions in MCI 3 (55%), and 6 conversions in MCI 4 (85%). AFT models were used to compare time until diagnosis of AD. MCI 2, MCI 3, and MCI 4 were all significantly different from MCI 1 in time to conversion but not from each other (Figure 4). There were three individuals who reverted back to the normal control diagnostic category at some time during follow-up (two from MCI 2 and one from MCI 1).

3.5. Secondary analyses

MCI 1 was clearly distinguishable from the rest of the MCI group, with a healthier biomarker profile, a lack of cognitive decline, and minimal conversions. This motivated a secondary analysis comparing MCI 1 to the ADNI normal controls. MCI 1 differed from the normal group at baseline on tests of memory (as expected because of the criteria for the MCI diagnostic category) but not on tests of executive function (Supplemental Table 1). The rate of annual change on cognitive tests in MCI 1 did not differ from the normal group (all $P > .10$), with the exception of Digit Span backward, in which MCI 1 performed worse (-0.32 additional points per year in MCI 1, $P = .02$). MCI 1 did not differ from normal controls in baseline values or annual change for total brain, ventricles, and entorhinal cortex (all $P > .25$). There were significant differences in baseline and annual change measures for the hippocampus, where MCI 1 began with smaller hippocampal volumes (approximately 5% smaller, $P = .02$) and experienced an atrophy rate approximately 2.5 times that of the normal controls ($P = .048$).

To better understand how MCI 2, MCI 3, and MCI 4 relate to one another, a secondary analysis was undertaken that excluded MCI 1 and took MCI 3 as the reference category. In general, the decrease in cognitive function in MCI 3 was significantly greater than MCI 2, but significantly smaller than MCI 4. This analysis was also performed on the longitudinal MRI measures in which MCI 3 and MCI 4 were not significantly different from each other for any of the measures except entorhinal cortex, where MCI 4 showed more rapid atrophy. MCI 2 showed significantly less atrophy than MCI 3 in all regions.

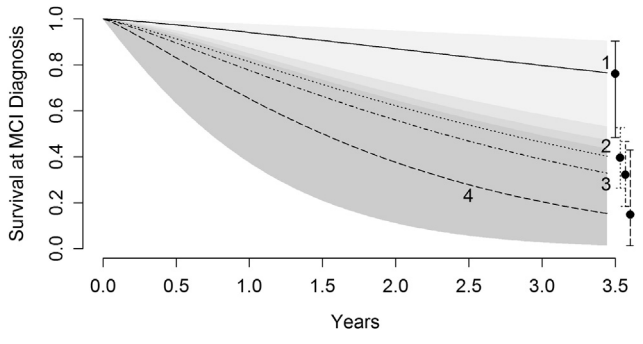

Fig. 4. "Survival" estimates from interval-censored accelerated failure time models, in which survival indicates maintaining a diagnosis of MCI rather than converting to AD. Model results indicate that MCI 2, 3, and 4 all differ significantly from MCI 1 but do not differ significantly among each other in time to conversion. MCI, mild cognitive impairment; AD, Alzheimer's disease.

4. Discussion

This study resulted in three key findings. First, there is biological heterogeneity at baseline among the ADNI amnestic MCI subjects despite being intentionally selected as a clinical phenotype often presumed to be the precursor to AD [26]. Second, a substantial portion of the individuals in this group (MCI 1) showed no clinical decline and was characterized by a remarkably healthy-looking biomarker profile. Third, a large cluster (MCI 3) failed to conform to the amyloid cascade hypothesis despite ultimately experiencing many conversions to AD.

The amyloid cascade hypothesis of AD suggests that the process begins with amyloid plaque deposition, followed in sequence by measurable changes in CSF tau proteins, then changes in brain volume, and finally clinically detectable cognitive change [4]. The goal of this study was to characterize heterogeneity within the ADNI MCI population—not to specifically test the veracity of the amyloid hypothesis [11]. Nonetheless, our findings are not entirely consistent with the proposed cascade of biological changes within this phenotypically refined cohort. Specifically, a large proportion of the subjects demonstrated biomarker abnormalities that appeared out of sequence relative to the model, and another group of subjects showed clinical memory deficits but lacked substantive biomarker abnormalities. Differences between the hypothesized sequence of events and our observations could happen for multiple reasons. First, it is likely that AD is not the sole cause of memory deficits in all subjects, despite attempting to recruit a homogenous group. For example, previous reports have identified a vascular subgroup of amnestic MCI subjects [27]. Furthermore, individuals may suffer with multiple pathologies that could modify the biomarkers or hypothesized sequence of pathological events [10]. It may also be possible that the amyloid cascade hypothesis describes the transition from memory loss to AD in some groups and the data in aggregate, but substantial variation in the sequence of biomarkers may be present among certain subgroups of amnestic individuals [1]. In fact, originators of the amyloid cascade hypothesis have since identified similar heterogeneity [28,29].

We found that MCI 1 had pronounced memory deficits that distinguished them from normal controls but had significantly fewer severe memory deficits than the rest of the MCI group. There was limited evidence to suggest that their memory impairment and smaller hippocampal volume was due to AD pathology. For example, CSF protein levels in this group were within normal limits, and

there was a slow or nonexistent rate of change in clinical function. These findings suggest that the individuals in MCI 1 are not in a temporary transition state to Alzheimer's dementia as presumed by the presence of impaired memory performance [26]. Whitwell et al. studied a similar group of stable amnestic MCI subjects (defined as no conversion to AD in 3-year follow-up after amnestic MCI diagnosis), comparing them both to cognitively normal subjects and amnestic MCI subjects who rapidly converted to AD. The stable MCI group had significantly smaller hippocampal volume than the normal controls, although there were not significant differences in gray matter loss [30]. Likewise, Wolk et al. found that 42% (8 of 19) of single-domain amnestic MCI subjects were amyloid-negative on Pittsburgh compound B amyloid imaging, supporting the notion of heterogeneity [31]. These findings may suggest that a non-AD process such as hippocampal sclerosis may be involved in the pathology of this subgroup of MCI [32], although other nonidentified causes—including congenital factors—may also explain these findings. Further studies of these subjects are clearly indicated.

MCI 2 and 4 seemed to follow the amyloid cascade hypothesis, with MCI 4 further along the trajectory than MCI 2. They had similarly low levels of $A\beta_{1\text{-}42}$ and had elevated tau proteins (Figure 2). MCI 4 had higher CSF tau levels; correspondingly, they had more severe atrophy and cognitive deficits. Despite entering the study with already severe levels of brain injury, MCI 4 continued to experience rapid change in regional brain volumes and cognitive abilities, and six of seven of the subjects in MCI 4 converted to AD within 3 years. Although these results should be interpreted with some caution because MCI 4 is a very small group, the clinical and biological differences between MCI 4 and the other clusters were substantial and favored a more severe state of neurodegeneration.

The biological profile of MCI 3 is the most inconsistent with the presumed sequence of biological markers leading to AD. For example, the $A\beta_{1\text{-}42}$ levels in MCI 3 were clearly abnormal, but they were in a range intermediate between normal and pathological levels commonly associated with Alzheimer's dementia [12]. In addition, average tau levels were essentially normal in MCI 3. This is in stark contrast to MCI 2 and 4, in which total tau means were approximately 1.5 and 6.5 SDs, respectively, above the normal controls. Moreover, there was no evidence for a second pathological process such as cerebrovascular brain injury to explain the biomarker findings. The hypothesized sequence of events prescribed by Jack et al. suggests that there should be substantial changes in tau before there are drastic changes in brain volume or cognitive function if the changes are due to AD [4]. MCI 3 had substantial brain atrophy, which was similar to the levels seen in the AD group, and it exhibited significantly lower cognitive function than MCI 1 and 2 at baseline and longitudinally.

Although it is not prominently discussed in the AD literature, there are other documented AD subgroups with minimal tau elevation. A recent CSF clustering paper separated AD subjects into three clusters, one of which had substantially lower tau and P-tau despite exhibiting a wide range of $A\beta_{1\text{-}42}$, which is similar to the pattern seen in MCI 3 [33]. Although the CSF assays in the Wallin et al. study differed from ADNI and therefore cannot be directly compared, the same group has performed other studies that included normal control subjects using the same assay. The total tau levels in their low-tau AD cluster (397 ± 113, $n = 87$) hardly differed from the total tau levels in aged normal controls in another study using the same assay (412 ± 232, $n = 34$) [34]. The normal controls in the aforementioned study likely contained some individuals who are in the early stages of AD and did not yet exhibit cognitive deficits, but this possibility was reduced by only including in the analysis controls that showed no substantive cognitive decline over a 4-year follow-up period. Another study examining subgroups of AD subjects using latent profile analysis found a similar group characterized by low tau [35]. This group was the oldest, approximately 76 years old, which is similar to our study.

Further research is necessary to determine whether the lack of congruence between the cascade model and the pathology in MCI 1 and 3 exists because the model is not suited to all cases of AD or because the cognitive deficits seen in MCI 1 and 3 are not due to AD. This question may be answerable in the future by examining longitudinal biomarker and cognitive data as well as neuropathological evidence from ADNI subjects who consented to postmortem examination.

Our data can also be viewed in light of the recently revised National Institute on Aging (NIA)/Alzheimer's Association (AA) diagnostic criteria for MCI due to AD [36]. According to these criteria, individuals with evidence of amyloid pathology (based on amyloid positron emission tomography [PET] imaging or CSF Aβ) and neuronal injury (based on fludeoxyglucose [FDG]-PET, CSF tau, or atrophy) have the greatest likelihood of MCI due to AD, whereas for those with conflicting biomarker information (e.g., low CSF Aβ but high CSF tau), the biomarkers are considered uninformative and the default clinical criteria hold. It is interesting to note that the smallest MCI clusters (1 and 4) are the ones that seem to correspond most consistently to these diagnostic criteria, because MCI 1 is generally negative for both categories of biomarkers whereas MCI 4 is generally positive. However, for a large proportion of the individuals in MCI 2 and 3, the biomarker measurements would be considered conflicting (and therefore uninformative) when, for example, CSF Aβ was abnormal but CSF tau (or atrophy) was normal. Even more troublesome are cases for which markers of neuronal injury conflict (e.g., the extensive atrophy and relatively normal CSF tau seen in MCI 3). This observation provides further support for the notion that the clinical phenotype of MCI is biologically heterogeneous [37].

The primary strength of this study is the use of unsupervised clustering without regard to cutoffs for dichotomous biomarker status, clinical outcomes, or longitudinal trajectories of biomarkers. Thus, any longitudinal patterns or clinical associations with cluster membership were not manufactured by the clustering process. The methodology is also a benefit in that it allows for an examination of multivariate structure in the data, which thrives on correlation between variables as opposed to being hindered by such correlation, as is the case with many regression methods. The primary weaknesses of this study are the limited number of subjects overall and the limited number of subjects with CSF fluid samples, which reduced the sample size available to simultaneously study CSF and imaging biomarkers. Another weakness is the fact that the clusters found were not compact and well separated but instead show overlap. Although this is to be expected in a biological system in which individuals are "moving" from cluster to cluster and may be exhibiting multiple pathologies simultaneously, it does leave the membership of individuals on the boundaries in some question. In this case, we view the use of cluster analysis not as a definitive classification method in which we sought to develop new categorical phenotypes, but as a tool for simultaneously using multiple biomarkers to understand the biological heterogeneity apparent with MCI subjects within the well-defined clinical phenotype of amnestic MCI. This analysis was exploratory in nature and would benefit from replication in other similar populations in which CSF and MRI measures are available to determine the extent to which the patterns found here are representative. Another limitation is the possibility that atrophy was nonlinear but it was modeled as linear; however, we believe that such nonlinearity is likely to be minimal over the short time frame.

The most important finding to come out of this analysis is the identification of biological and cognitive heterogeneity within the presumed homogenous clinical phenotype of amnestic MCI. Furthermore, these findings are relevant not only to the understanding of biological processes leading to memory loss, but also to clinical trial methodology. For example, in subjects such as those in MCI 1 (14% of the subjects) whose memory deficits placed them in the MCI diagnostic group but who exhibited very little change over time, their slow rate of change and the likelihood that their deficits were not clearly related to AD would make them poor candidates for inclusion in clinical trials. Likewise, individuals with profiles matching MCI 3, which made up 37% of the subjects, may not be suitable for inclusion in treatment trials that would emphasize reductions in tau as a treatment outcome. It is important to note that these two groups combined made up over 50% of the subjects in this analysis. If ADNI had been running a clinical trial specific to tau-mediated brain injury in the MCI diagnostic group, the inclusion of MCI 1 and MCI 3 could have resulted in a substantial loss of power to detect beneficial effects of treatment.

In conclusion, our findings indicate that the clinical phenotype of amnestic MCI is biologically and behaviorally heterogeneous, and a more complete understanding of this heterogeneity will not only improve our understanding of transition phases from normal cognitive aging, but it will also likely benefit the design and implementation of clinical trials aimed at treatment of the earliest pathological changes associated with AD.

Acknowledgments

Data collection and sharing for this project was funded by ADNI (National Institutes of Health [NIH] Grant U01 AG024904). ADNI is funded by NIA, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: Abbott; AA; 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 is providing 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 is 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 R01 AG021028 and UL1 TR0002.

Conflict of interest: None reported for J.N. C.D. is a member of the MRI core and L.B. leads the biostatistics core of ADNI. Each receives research support from ADNI for these services. S.L. works with the ADNI PET core, receives ADNI research support, and has received consulting fees from Avid Radiopharmaceuticals and Biogen Idec.

Data used in preparation of this article were obtained from the ADNI database (adni.loni.ucla.edu). As such, the investigators within ADNI contributed to the design and implementation of ADNI and/or provided data, but they 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.

RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the literature related to the sequence of biomarker changes in AD, levels of AD biomarkers in MCI, and the pathology underlying MCI using Google Scholar and PubMed. Relevant sources have been cited.
2. Interpretation: Our results augment a growing body of literature pointing away from the characterization of amnestic MCI as prodromal AD and demonstrate evidence of considerable biological heterogeneity, potentially the result of comorbid neuropathology. Our results also demonstrate a potential complication in applying the latest NIA/AA diagnostic criteria for MCI due to AD in that we have identified a large subgroup with conflicting biomarkers for neuronal injury (extensive atrophy and relatively normal CSF tau levels) despite many conversions to AD during follow-up. This possibility is acknowledged, but it is not directly addressed in the NIA/AA criteria.
3. Future directions: Simultaneous examination of MRI and CSF biomarkers in other study populations will be necessary to determine whether subgroups with heterogeneous biomarker profiles are consistently identified, particularly outside of clinic-based samples. Further study including longer-term follow-up and measures of underlying processes, either by PET imaging or postmortem, will be required to understand the connection between these biomarker profiles and the underlying neuropathology.

References

[1] Nettiksimmons J, Harvey D, Brewer J, Carmichael O, DeCarli C, Jack CR Jr, et al. Subtypes based on cerebrospinal fluid and magnetic resonance imaging markers in normal elderly predict cognitive decline. Neurobiol Aging 2010;31:1419–28.
[2] Dubois B, Albert ML. Amnestic MCI or prodromal Alzheimer's disease? Lancet Neurol 2004;3:246–8.
[3] Jicha GA, Parisi JE, Dickson DW, Johnson K, Cha R, Ivnik RJ, et al. Neuropathologic outcome of mild cognitive impairment following progression to clinical dementia. Arch Neurol 2006;63:674–81.
[4] Jack CR Jr, Knopman DS, Jaqust WJ, Shaw LM, Aisen PS, Weiner MW, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol 2010; 9:119–28.
[5] Ganguli M, Dodge HH, Shen C, DeKosky ST. Mild cognitive impairment, amnestic type: an epidemiologic study. Neurology 2004; 63:115–21.
[6] Zanetti M, Ballabio C, Abbate C, Cutaia C, Vergani C, Bergamaschini L. Mild cognitive impairment subtypes and vascular dementia in community-dwelling elderly people: a 3-year follow-up study. J Am Geriatr Soc 2006;54:580–6.
[7] Yaffe K, Petersen RC, Lindquist K, Kramer J, Miller B. Subtype of mild cognitive impairment and progression to dementia and death. Dement Geriatr Cogn Disord 2006;22:312–9.
[8] Caroli A, Testa C, Geroldi C, Nobili F, Barnden LR, Guerra UP, et al. Cerebral perfusion correlates of conversion to Alzheimer's disease in amnestic mild cognitive impairment. J Neurol 2007;254:1698–707.
[9] Dickson DW, Davies P, Bevona C, Van Hoeven KH, Factor SM, Grober E, et al. Hippocampal sclerosis: a common pathological feature of dementia in very old (> or = 80 years of age) humans. Acta Neuropathol 1994;88:212–21.
[10] Schneider JA, Arvanitakis Z, Bang W, Bennett DA. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. Neurology 2007;69:2197–204.
[11] Petersen RC, Aisen PS, Beckett LA, Donohue MC, Garnst AC, Harvey DJ, et al. Alzheimer's Disease Neuroimaging Initiative (ADNI): clinical characterization. Neurology 2010;74:201–9.
[12] Jack CR Jr, Bernstein MA, Fox NC, Thompson P, Alexander G, Harvey D, et al. The Alzheimer's Disease Neuroimaging Initiative (ADNI): MRI methods. J Magn Reson Imaging 2008;27:685–91.
[13] Holland D, Dale AM, Alzheimer's Disease Neuroimaging Initiative. Nonlinear registration of longitudinal images and measurement of change in regions of interest. Med Image Anal 2011;15:489–97.
[14] Laird NM, Ware JH. Random-effects models for longitudinal data. Biometrics 1982;38:963–74.
[15] Schwarz C, Fletcher E, DeCarli C, Carmichael O. Fully-automated white matter hyperintensity detection with anatomical prior knowledge and without FLAIR. Inf Process Med Imaging 2009;21:239–51.
[16] Shaw LM. PENN biomarker core of the Alzheimer's disease Neuroimaging Initiative. Neuro-Signals 2008;16:19–23.
[17] Wechsler D. Wechsler Memory Scale–Revised. San Antonio, TX: Psychological Corporation; 1987.
[18] Wechsler D. Wechsler Adult Intelligence Scale–Revised. San Antonio, TX: Psychological Corporation; 1981.
[19] Partington JE. Partington's Pathway Test. Psych Serv Center J 1949; 1:9–20.
[20] Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology 1989; 39:1159–65.
[21] Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–98.
[22] Mohs RC. Administration and Scoring Manual for the Alzheimer's Disease Assessment Scale, 1994 Revised Edition. New York: The Mount Sinai School of Medicine; 1994.
[23] Rey A. L'Examen Clinique en Psychologie (Psychological Clinical Examination). Oxford, United Kingdom: Presses Universitaries De France; 1964.
[24] Tibshirani R, Walther G, Hastie T. Estimating the number of clusters in a data set via the gap statistic. J Royal Stat Soc B Stat Methodol 2001; 63:411–23.
[25] R Development Core Team. A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2012.
[26] Morris JC, Cummings J. Mild cognitive impairment (MCI) represents early-stage Alzheimer's disease. J Alzheimers Dis 2005;7:235–9. discussion 255–62.
[27] Nordahl CW, Ranganath C, Yonelinas AP, DeCarli C, Reed BR, Jaqust WJ. Different mechanisms of episodic memory failure in mild cognitive impairment. Neuropsychologia 2005;43:1688–97.
[28] Jack CR Jr, Knopman DS, Weigand SD, Wiste HJ, Vemuri P, Lowe V, et al. An operational approach to National Institute on Aging-

Alzheimer's Association criteria for preclinical Alzheimer disease. Ann Neurol 2012;71:765–75.

[29] Knopman DS, Jack CR Jr, Wiste HJ, Weigand SD, Vemuri P, Lowe VJ, et al. Brain injury biomarkers are not dependent on beta-amyloid in normal elderly. Ann Neurol 2012; http://dx.doi.org/10.1002/ana.23816 [Epub ahead of print].

[30] Whitwell JL, Shiung MM, Przybelski SA, Weigand SD, Knopman DS, Boeve BF, et al. MRI patterns of atrophy associated with progression to AD in amnestic mild cognitive impairment. Neurology 2008; 70:512–20.

[31] Wolk DA, Price JC, Saxton JA, Snitz BE, James JA, Lopez OL, et al. Amyloid imaging in mild cognitive impairment subtypes. Ann Neurol 2009;65:557–68.

[32] Zarow C, Sitzer TE, Chui HC. Understanding hippocampal sclerosis in the elderly: epidemiology, characterization, and diagnostic issues. Curr Neurol Neurosci Rep 2008;8:363–70.

[33] Wallin AK, Blennow K, Zetterberg H, Londos E, Minthon L, Hansson O. CSF biomarkers predict a more malignant outcome in Alzheimer disease. Neurology 2010;74:1531–7.

[34] Buchhave P, Blennow K, Zetterberg H, Stomrud E, Londos E, Andreasen N, et al. Longitudinal study of CSF biomarkers in patients with Alzheimer's disease. PloS One 2009;4:e6294.

[35] Iqbal K, Flory M, Khatoon S, Soininen H, Pirttila T, Lehtovirta M, et al. Subgroups of Alzheimer's disease based on cerebrospinal fluid molecular markers. Ann Neurol 2005;58:748–57.

[36] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:270–9.

[37] DeCarli C. Mild cognitive impairment: prevalence, prognosis, aetiology, and treatment. Lancet Neurol 2003;2:15–21.

Supplemental Table 1
Baseline demographic, genetic, clinical, and biomarker measures for the ADNI normal control group, MCI participants not included in this analysis because of missing data, MCI participants included in this analysis, and the ADNI AD group

	Normal controls	Nonclustered MCI	Clustered MCI	AD
n	222	244	138	181
Age (years)	76.0 (5.0)	75.2 (7.4)	74.0 (7.4)	75.1 (7.6)
Gender (male)	52%	65%	62%	53%
Education (years)	16.0 (2.8)	15.4 (3.1)	16.0 (2.9)	14.8 (3.1)
APOE (∈4 carrier)	27%	54%	54%	67%
Parent with AD	26%	24%	29%	24%
Follow-up (years)	2.8 (0.6)	2.4 (0.9)	2.6 (0.6)	1.8 (0.6)
FAQ	0.1 (0.6)	3.9 (4.5)	3.7 (4.4)	12.9 (6.8)
Geriatric depression	0.9 (1.1)	1.6 (1.4)	1.6 (1.3)	1.6 (1.4)
Digit Span forward	8.8 (2)	8.2 (2)	8.2 (2.1)	7.6 (1.9)
Digit Span backward	7.2 (2.2)	6.1 (2.1)	6.4 (2)	5 (1.8)
Digit Symbol	45.8 (10.3)	36.7 (11.1)	37.3 (11.1)	27.1 (12.8)
Trails B	89.7 (44.7)	130.9 (74.3)	130 (70.9)	197.1 (86.7)
Category Naming	34.6 (8.1)	26.3 (7.4)	27.3 (7.6)	20.4 (7.2)
MMSE	29.1 (1)	27.1 (1.7)	26.9 (1.8)	23.4 (2)
ADAS-cog	6.2 (2.9)	11.5 (4.4)	11.7 (4.5)	18.5 (6.4)
RAVLT (sum of five trials)	43.5 (8.9)	31.1 (9.4)	30.2 (8.6)	23.3 (7.5)
RAVLT 30 min	7.5 (3.7)	3 (3.4)	2.6 (3.2)	0.8 (1.6)
RAVLT (% savings)	0.7 (0.3)	0.3 (0.3)	0.3 (0.3)	0.1 (0.2)
Logical Memory II	13 (3.6)	3.9 (2.6)	3.6 (2.7)	1.3 (1.9)
Whole brain volume*	0.6844 (0.0249)	0.6729 (0.0275)	0.6737 (0.0253)	0.6604 (0.0264)
Hippocampal volume*	0.0050 (5e-04)	0.0045 (7e-04)	0.0043 (6e-04)	0.0041 (7e-04)
Ventricle volume*	0.0257 (0.0125)	0.0297 (0.0131)	0.0298 (0.0126)	0.0344 (0.0144)
Entorhinal thickness†	6.500 (0.610)	5.88 (0.978)	5.824 (0.871)	5.183 (0.887)
WMH‡	2.651 (2.477)	2.795 (2.772)	2.434 (2.014)	3.922 (7.32)
$A\beta_{1\text{-}42}$§	206.2 (55.3)	175.0 (60.5)	159.5 (52.6)	142.9 (41.2)
Tau§	68.9 (28.1)	100.8 (59.8)	102.0 (51.9)	119.5 (56.1)
$P\text{-}tau_{181}$§	24.5 (13.6)	32.6 (16.2)	36.1 (17.3)	41.0 (19.5)
P-tau/Aβ	0.38 (0.25)	0.72 (0.65)	0.74 (0.52)	0.91 (0.48)
Tau/Aβ	0.14 (0.12)	0.23 (0.18)	0.26 (0.17)	0.32 (0.19)
Homocysteine‖	9.98 (2.79)	10.86 (2.83)	10.19 (2.84)	10.63 (3.16)

Abbreviations: CSF, cerebrospinal fluid; MCI, mild cognitive impairment; AD, Alzheimer's disease; *APOE*, apolipoprotein E; FAQ, Functional Activity Questionnaire; MMSE, Mini-Mental State Examination; ADAS-cog, Alzheimer's Disease Assessment Scale - cognitive subscale; RAVLT, Rey's Auditory Vocabulary List Test; WMH, white matter hyperintensities; P-tau, phosphorylated tau; Aβ, amyloid-β.

NOTE. Means and standard deviations are reported unless otherwise noted. It should be noted that the CSF measures reported are based on approximately half of the stated sample size in the normal control and AD groups. CSF measures in the nonclustered MCI group are based on 51 individuals who were excluded from clustering because of missing values in other variables.

*Presented as fraction of intracranial volume.

†Average of right and left in millimeters.

‡Cubic centimeters.

§Picograms per milliliter.

‖Micromoles per liter.