Increased Brain Atrophy Rates in Cognitively Normal Older Adults with Low Cerebrospinal Fluid Aβ1-42

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Objective: To identify cognitively normal individuals at risk of Alzheimer disease (AD) based on cerebrospinal fluid (CSF) Aβ1-42, and to determine rates of cerebral atrophy.
Methods: Control subjects from the Alzheimer's Disease Neuroimaging Initiative with CSF and serial magnetic resonance imaging (MRI) were dichotomized on CSF Aβ1-42 (normal control [NC]-high >192pg/ml; NC-low $\leq$192pg/ml). Baseline and 1-year MRIs were registered, and brain, hippocampal, and ventricular volumes and annualized volume changes were calculated. Baseline characteristics, CSF profiles, neuropsychology, brain volumes and atrophy rates, and *APOE*, *PICALM*, *CLU*, and *TOMM40* genotypes were compared. Sample sizes to power presymptomatic clinical trials based on rate of atrophy were calculated.
Results: Forty of 105 (38%) were classified as NC-low, and 65 (62%) as NC-high. There were no differences in age (76.3 $\pm$ 5.1 vs 74.9 $\pm$ 5.1 years), gender, brain volumes, and all but 1 cognitive score (Trails B; $p = 0.015$). The NC-low group had higher tau ($p = 0.005$) and p-tau ($p < 0.001$), and was more likely to be *APOE4* positive (48% vs 11%, $p < 0.001$). The NC-low group had significantly higher whole brain loss (9.3 vs 4.4ml/yr, $p < 0.001$), ventricular expansion (2.04 vs 0.95ml/yr, $p = 0.002$), and hippocampal atrophy rate (0.07 vs 0.03ml/yr, $p = 0.029$). Baseline Aβ1-42 level was strongly correlated with rate of brain atrophy only in the NC-low group ($p < 0.001$). Using 141 (95% confidence interval, 86–287) patients per arm provides 80% power in a 1-year treatment trial to show 25% slowing of brain atrophy in the NC-low group.
Interpretation: A significant percentage of healthy older adults have CSF profiles consistent with AD and increased rates of brain atrophy, suggesting that they may be in the earliest stages of neurodegeneration. Brain atrophy may be a feasible outcome measure for AD prevention studies.

ANN NEUROL 2010;68:825–834

Alzheimer disease (AD) is rapidly increasing in prevalence as the population ages[1]; by 2050, annual care costs in the United States may exceed $1 trillion.[2] There is an urgent need to develop treatments to slow or halt disease progression, and numerous potentially disease-modifying agents are in development. Delaying disease onset by 5 years has been estimated to halve the prevalence and care costs of AD.[2]

The defining pathological features of AD are deposition of fibrillar β-amyloid and hyperphosphorylated tau, and neuronal cell loss leading to excess brain atrophy.[3] These events are hypothesized to follow a predictable sequence and predate symptoms.[4] When episodic memory impairment is significant, amnestic mild cognitive impairment (MCI)[5] can be diagnosed; once cognitive impairment impacts on daily living, a clinical diagnosis of AD can be made.[6]

Although slowing of cognitive decline remains the gold standard outcome measure for clinical trials, disease-specific biomarkers, including in vivo measures of

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.22315

Received Aug 26, 2010, and in revised form Oct 17, 2010. Accepted for publication Oct 22, 2010.

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 analysis or writing of this report. A full list of ADNI investigators is available at: http://www.loni.ucla.edu/ADNI/Collaboration/ADNI_Manuscript_Citations.pdf

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amyloid and tau using cerebrospinal fluid (CSF) markers[7] and positron emission tomography (PET) ligands,[8] brain hypometabolism using fluorodeoxyglucose-PET,[9] and brain atrophy rate derived from serial magnetic resonance imaging (MRI)[10] are increasingly used in an attempt to distinguish disease-modifying from symptomatic effects.[11]

The prospect of disease modification has intensified the need to diagnose very early AD with high accuracy. The ultimate goal is to identify and treat asymptomatic individuals with prodromal AD, or those at high risk of developing the disease.[12–14] By definition, such individuals will be asymptomatic, and disease biomarkers or high-risk traits will be required for identification. For presymptomatic treatment trials, demonstration of disease modification will ultimately require evidence of delay to symptom onset or conversion to AD. Supportive evidence of disease modification may be demonstrable over shorter time frames using disease biomarkers.[12] How best to design presymptomatic AD treatment trials is of intense current interest, with the Alzheimer's Prevention Initiative aiming to determine methods for evaluating and gaining regulatory approval for promising treatments.[12]

Proposed approaches for presymptomatic studies include identifying individuals destined to get AD on the basis of carrying an autosomal dominant mutation, those at increased genetic risk for sporadic AD because they carry an *APOE4* allele, and those with biomarkers suggestive of preclinical AD (eg, evidence of amyloid deposition on PET imaging or low CSF Aβ1-42).[12] In this study, we aimed to determine the feasibility of presymptomatic treatment studies in AD using serial MRI as an outcome measure by dichotomizing normal elderly individuals on the basis of low CSF Aβ1-42.

Subjects and Methods

Subjects

All subjects were drawn from the Alzheimer's Disease Neuroimaging Initiative (ADNI), a multicenter publicly/privately funded longitudinal study investigating adult subjects with AD, amnestic MCI, and normal cognition. Participants undergo baseline and periodic clinical and neuropsychometric assessments and serial MRI. Approximately 60% have CSF, and a subset have PET imaging. Details are available at http://www.adni-info.org. Written informed consent was obtained, as approved by the institutional review board at each of the participating centers.

We downloaded data from LONI (http://adni.loni.ucla.edu) and included all control subjects who had baseline CSF and usable 1.5T MRI imaging at baseline and 1 year. All subjects had a standardized cognitive assessment at baseline (details at www.adni-info.org/Scientists/Pdfs/adniproceduresmanual12.pdf), which included: Mini Mental State Examination (MMSE), Clinical Dementia Rating–Sum of Boxes (CDR-SB) Alzheimer's Disease Assessment Scale, Cognitive Subscale (ADAS-Cog; 13 point scale), Neuropsychiatric Inventory (NPI) score, Auditory Verbal Learning Test (AVLT) delayed recall, verbal fluency, and Trails A and B. Blood was drawn for homocysteine quantification and genetic analysis. CSF measures of tau, p-tau, and Aβ1-42 were performed centrally, as previously described.[15] To assess whether individuals converted to a clinical diagnosis of MCI or AD, we interrogated clinical records up to and including the visit 36 months from baseline, available on the LONI website on August 12, 2010.

Genetics

Details of the genotyping methods have been published.[16] For each individual, we downloaded *APOE* genotype and single nucleotide polymorphism (SNP) data from the LONI website. We extracted data for 4 SNPs of interest, based on prior studies: rs2075650 (*TOMM40*),[17] rs11136000 (*CLU*), rs1408077 (*CR1*), and rs3851179 (*PICALM*).[18]

MRI

Details of the MRI methodology have previously been described.[19] In brief, MRI was performed using standardized protocols on 1.5T MRI units. MRI protocols included the acquisition of high-resolution volumetric T1-weighted, inversion-recovery (IR)-prepared structural images. Postprocessing steps included corrections for distortion due to gradient nonlinearity and for image intensity nonuniformity, and scalings based on phantom measures.

Local image analysis was performed using the MIDAS software package.[20] Semiautomated whole brain and ventricular segmentation was performed. Change (in milliliters) over time was obtained using the boundary shift integral (BSI) following a 9 degrees of freedom registration and differential bias correction of the follow-up to baseline scans. Volume change was measured for ventricles using the BSI (UBSI), and for whole brain using the KN-BSI method.[21] Total intracranial volume was generated using SPM8.[21] Hippocampal volume was measured using the automated HMAPS method, and changes over time were quantified using the BSI.[22]

Statistical Methods

A previous CSF study from a group of patients with autopsy-confirmed AD analyzed with identical methodology estimated that a CSF Aβ1-42 cutoff of 192pg/ml was the best discriminator of AD from controls.[15] We therefore divided the ADNI control subjects into CSF Aβ1-42 $>$192pg/ml (normal control [NC]-high) and $\leq$192pg/ml (NC-low). Baseline characteristics, cross-sectional brain volume, and 1-year atrophy rates were compared between groups using 2-sample t tests, allowing for unequal variances. The proportions of cases and controls with zero, 1, or 2 risk alleles were compared for each SNP of interest using Fisher's exact test, and odds for having a minor allele were compared between the groups.

CSF results were compared to published results from autopsy-confirmed AD cases.[15] Linear regression models were fitted to explore the association between atrophy rates and CSF Aβ1-42, and between cognitive tests and CSF Aβ1-42, tau, and p-tau. Further regression models were fitted that allowed for a shift in mean atrophy rate and change in slope at CSF Aβ1-42 = 192pg/ml to assess whether the association between atrophy and CSF Aβ1-42 differed in the NC-low and NC-high groups. Robust standard errors were used to allow for possible heteroscedasticity. Mean atrophy rates were compared between *APOE4*-negative and *APOE4*-positive subjects using 2-sample *t* tests.

We assessed the CSF, imaging, and clinical characteristics of individuals who had a change in clinical diagnosis during follow-up. Using data from the NC-low group, we used the standard formula to estimate sample sizes needed to provide 80% power with 5% type 1 error to detect a 25% absolute reduction in annualized rate of whole brain, ventricular, or hippocampal change; and to express the 25% absolute reduction as a proportion of the NC-low to NC-high group difference.[20] Similar calculations were performed in the *APOE4*-positive group. Bias-corrected and accelerated 95% bootstrap confidence intervals (CIs) were found for sample size estimates (100,000 bootstrap samples). All analyses were performed using Stata 11.1 (Stata Corp, College Station, TX).

Results

Baseline Group Characteristics

DEMOGRAPHICS AND COGNITIVE SCORES. Dividing the control group by CSF Aβ-42, 40/105 (38%) subjects were defined as NC-low (Aβ-42 $\leq$192) and 65/105 (62%) as NC-high. Demographics are shown in Table 1. There was no evidence of differences in baseline age, gender, blood pressure, homocysteine level, or NPI score between the groups. Similarly, there was no evidence of differences in mean MMSE, CDR-SB, ADAS-Cog, AVLT delayed recall, category fluency, or Trails A time. NC-low individuals were statistically significantly slower on the Trails B task ($p = 0.015$). Regression analyses confirmed a statistically significant linear association between Trails B and, tau ($p = 0.026$), and p-tau ($p = 0.048$), and weak evidence for CSF Aβ1-42 ($p = 0.083$).

CSF PROFILES. There were highly statistically significant differences in CSF profiles between the groups. The NC-low group not only had lower Aβ1-42 (by definition), but also higher total tau, p-tau, tau/Aβ1-42 ratio, and p-tau/Aβ1-42 ratio than the NC-high group ($p < 0.001$). Comparing these CSF results with autopsy-confirmed AD reference ranges[15] demonstrated that for Aβ1-42, levels were lowest in the postmortem-proven AD, intermediate in NC-low, and highest in the NC-high group (Table 2). The opposite pattern was seen for tau, p-tau, tau/Aβ1-42 ratio, and p-tau/Aβ1-42 ratio. Results in the postmortem-proven group were statistically significantly different from both NC groups for all measures, apart from Aβ1-42, p-tau, and p-tau/Aβ1-42 ratio, which were not significantly different from the NC-low group. Using previously defined cutoffs, as well as being separated by Aβ1-42, individuals in the NC-low group were significantly more likely to be classified within the AD range than those in the NC-high group for p-tau (53% vs 25%; $p = 0.006$), tau/Aβ1-42 ratio (75% vs 12%; $p < 0.001$), and p-tau/Aβ1-42 ratio (85% vs 25%; $p < 0.001$), with tau reaching borderline significance (27.5% vs 12.3%, $p = 0.068$).

GENETICS. *CR1* data were not available for 2 individuals. There was a highly statistically significant difference in *APOE* genotype between the groups, with 19/40 (48%) of the NC-low group possessing $\geq$1 *APOE4* allele, compared with only 7/65 (11%) of the NC-high group ($p < 0.001$). There was statistically significant evidence of a difference in the distribution of the risk allele of the *TOMM40* gene (rs2075650) between the groups ($p = 0.007$). However, a further logistic regression analysis showed that after adjusting for *APOE4* positivity, there was no evidence ($p = 0.49$) of an independent effect of differential allelic distribution for the *TOMM40* gene. This occurred because *APOE4* positivity was strongly associated with possession of 1 or more TOMM40 risk alleles ($p < 0.001$), and the former was more strongly associated with low Aβ1-42 than the latter. There were no statistically significant differences in the distribution of alleles for *CLU, CR1,* or *PICALM* genes (see Table 1). Odds ratios for possession of the minor allele (95% CI) were: *TOMM40* = 2.85 (1.31–6.22), *CLU* = 0.96 (0.55–1.68), *CR1* = 0.88 (0.40–1.92), and *PICALM* = 0.67 (0.37–1.21).

BASELINE BRAIN VOLUMES. There were no statistically significant differences in baseline brain, ventricular, or hippocampal volumes (with or without adjustment for total intracranial volume or brain volume) between the 2 groups.

Change Over Time

ATROPHY RATES. Over 1 year, the NC-low group had significantly higher rates of whole brain atrophy (9.3 vs 4.4ml/yr, $p < 0.001$), ventricular expansion (2.04 vs 0.95ml/yr, $p = 0.002$), and hippocampal atrophy (0.07 vs 0.03ml/yr, $p = 0.029$).

Plots of atrophy rates against baseline Aβ1-42 are shown in the Fig. For the whole group, there was strong evidence ($p < 0.001$) that lower Aβ1-42 was associated

TABLE 1: Baseline Demographics, Genotypes, Neuropsychometry, CSF Profiles, Brain Volumes, and 1-Year Rates of Atrophy in NC-low and NC-high Groups

Characteristic	NC-low, CSF Aβ1–42 ≤192, n=40		NC-high, CSF Aβ1–42 >192, n=65		
	Mean ± SD	95% CI	Mean ± SD	95% CI	*p*
Age, yr	76.3 ± 5.1	74.7–78.0	74.9 ± 5.1	73.7–76.2	0.18
Gender, % male	55.0%	38.5%–70.7%	50.8%	38.1%–63.3%	0.69
Systolic blood pressure, mmHg	131.5 ± 15.1	126.6–136.3	132.3 ± 17.8	127.9–136.7	0.79
Diastolic blood pressure, mmHg	74.0 ± 9.4	70.9–77.0	74.5 ± 8.1	72.5–76.5	0.76
Baseline homocysteine (μmol/L)	10.1 ± 2.4	9.3–10.5	10.0 ± 3.0[a]	9.2–10.7	0.86
*APOE*4 positive (%)	47.5%	31.5%–63.9%	10.8%	4.4%–20.9%	<0.001
rs2075650 (*TOMM40*) minor allele 0:1:2, %	57.5%:40.0%:2.5%		83.1%:15.4%:1.5%		0.007
rs3851179 (*PICALM*) minor allele 0:1:2, %	50.0%:42.5%:7.5%		36.9%:50.8%:12.3%		0.42
rs1408077 (*CR1*) minor allele 0:1:2, %	71.1%:29.0%:0%[b]		69.2%:29.2%:1.5%		1.00
rs11136000 (*CLU*) minor allele 0:1:2, %	40.0%:37.5%:22.5%		30.8%:53.9%:15.4%		0.26
Neuropsychiatric Inventory score	0.18 ± 0.45	0.03–0.32	0.31 ± 0.75	0.12–0.49	0.26
MMSE	29.2 ± 0.9	28.9–29.5	29.0 ± 1.1	28.7–29.3	0.39
CDR-SB	0.01 ± 0.08	−0.01–0.04	0.03 ± 0.12	0.00–0.06	0.35
ADAS-Cog	10.6 ± 4.1	9.3–11.9	9.1 ± 4.2	8.1–10.2	0.08
AVLT-delayed recall	7.8 ± 3.2	6.8–8.8	8.0 ± 3.2	7.2–8.8	0.80
Category fluency (vegetables)	14.0 ± 3.9	12.7–15.2	14.7 ± 3.7	13.8–15.7	0.31
Category fluency (animals)	19.6 ± 5.7	17.8–21.4	19.4 ± 6.0	18.0–20.9	0.91
Trails test (A)	38.3 ± 13.9	33.9–42.7	35.1 ± 11.1	32.3–37.8	0.22
Trails test (B)	101.9 ± 55.4	84.1–119.6	78.6 ± 22.8	72.9–84.2	0.015
Baseline Aβ1–42, pg/ml	142.5 ± 26.9	133.9–151.1	242.8 ± 26.0	236.4–249.3	NA
Baseline tau, pg/ml	80.4 ± 33.7	69.6–91.1	63.0 ± 21.3	57.7–68.3	0.005
Baseline p-tau, pg/ml	31.8 ± 18.1	26.0–37.5	20.5 ± 7.8	18.6–22.4	0.005
Baseline tau/Aβ1–42 ratio	0.59 ± 0.30	0.49–0.69	0.26 ± 0.09	0.24–0.29	<0.001
Baseline p-tau/Aβ1–42 ratio	0.238 ± 0.163	0.186–0.290	0.085 ± 0.032	0.077–0.093	<0.001
Baseline brain volume, ml	1,077.4 ± 105.0	1,043.9–1111.0	1,054.2 ± 102.8	1,028.7–1079.7	0.27
Baseline ventricular volume, ml	39.4 ± 16.2	34.2–44.5	35.8 ± 20.9	30.7–41.0	0.34
Baseline hippocampal volume, ml	5.17 ± 0.62	4.97–5.37	5.26 ± 0.70	5.09–5.44	0.47
Whole brain atrophy rate, ml/yr	9.3 ± 6.9	7.0–11.5	4.4 ± 5.3	3.1–5.7	<0.001
Ventricular expansion rate, ml/yr	2.04 ± 1.93	1.42–2.66	0.95 ± 1.14	0.67–1.23	0.002
Hippocampal atrophy rate, ml/yr	0.071 ± 0.097	0.040–0.103	0.030 ± 0.087	0.008–0.051	0.029

[a]n = 64; [b]n = 38.

NC = normal control; CSF = cerebrospinal fluid; SD = standard deviation; CI = confidence interval; MMSE = Mini Mental State Examination; CDR-SB = Clinical Dementia Rating–Sum of Boxes; ADAS-Cog = Alzheimer's Disease Assessment Scale, Cognitive Subscale; AVLT = Auditory Verbal Learning Test; NA = not applicable.

with higher rates of brain atrophy. This relationship was present within the NC-low group ($p = 0.005$), but not the NC-high group ($p = 0.65$), and a test for interaction showed strong evidence ($p = 0.009$) that the slopes differ between the 2 groups. There was no evidence ($p = 0.18$) that mean brain atrophy differed according to *APOE4* status. Lower Aβ1-42 was associated with increased rates of ventricular expansion across the whole group ($p < 0.001$), with a relationship remaining in the NC-low group ($p = 0.002$) but not seen in the NC-high group ($p = 0.37$). Again, there was strong evidence ($p = 0.007$) for differential slopes in the NC-low and NC-high groups. *APOE4* positivity was associated with increased rates of ventricular expansion across the whole group ($p = 0.018$), but this effect was not seen in either NC-low or NC-high subgroups, and was no longer significant ($p = 0.07$) when Aβ1-42 was accounted for. There was evidence that lower Aβ1-42 was associated with increased hippocampal atrophy rate over the group as a whole ($p = 0.009$), which was still statistically significant in the NC-low group ($p = 0.046$), but not in the NC-high group ($p = 0.75$). There was weak evidence that the slope of the association differed between the 2 groups ($p = 0.071$). There was no evidence for an influence of *APOE4* status on hippocampal atrophy rate ($p = 0.36$).

SAMPLE SIZE CALCULATIONS. Recruiting NC-low patients to power a 1-year treatment trial to detect an absolute 25% slowing of brain atrophy rate (equivalent to ~48% slowing if the maximum possible loss was equal to mean loss in the NC-high group), 141 (95% CI, 86–287) subjects would be required per arm using whole brain atrophy, 225 (95% CI, 147–442) using ventricular expansion, and 467 (95% CI, 197–2675) using hippocampal atrophy rate. Selecting individuals on the basis of *APOE4* positivity alone reduces the population available for recruitment (in this cohort to ~25%). To detect an absolute 25% slowing of rates (~60–70% slowing accounting for the *APOE4*-negative group) would require an estimated 224 (95% CI, 118–575) patients per arm for whole brain atrophy, 222 (95% CI, 135–431) for ventricular expansion, and 703 (95% CI, 211 to >40,000) for hippocampal atrophy. There was no evidence that these differed from the corresponding sample sizes based on selection according to CSF Aβ1-42.

CHANGE IN CLINICAL DIAGNOSIS. To date, 5 control individuals have converted to MCI, and 1 to AD according to data downloaded from the ADNI LONI website (Table 3). Of these, we had classified 4 as NC-low, although 1 had a very borderline Aβ1-42

TABLE 2: CSF Profiles in the NC-low and NC-high Groups with Profiles and AD/Control Cutoffs from Autopsy-Confirmed AD Subjects with CSF Analyzed Using the Same Methodology[15]

Profile	PM-Proven AD, n = 56[a]			NC-Low, n = 40			NC-High, n = 65		
	Mean ± SD	95% CI	Cutoff Value	Mean ± SD	95% CI	*p*	Mean ± SD	95% CI	*p*
Baseline Aβ1–42, pg/ml	132 ± 34	123–141	<192	142.5 ± 26.9	133.9–151.1	0.09	242.8 ± 26.0	236.4–249.3	<0.001
Baseline tau, pg/ml	135 ± 95	110–161	>93	80.4 ± 33.7	69.6–91.1	<0.001	63.0 ± 21.3	57.7–68.3	<0.001
Baseline p-tau, pg/ml	39 ± 29	31–47	>23	31.8 ± 18.1	26.0–37.5	0.14	20.5 ± 7.8	18.6–22.4	<0.001
Baseline tau/Aβ1–42 ratio	1.1 ± 1.0	0.9–1.4	>0.39	0.59 ± 0.30	0.49–0.69	<0.001	0.26 ± 0.09	0.24–0.29	<0.001
Baseline p-tau/Aβ1–42 ratio	0.3 ± 0.2	0.2–0.4	>0.1	0.238 ± 0.163	0.186–0.290	0.10	0.085 ± 0.032	0.077–0.093	<0.001

p values are from 2-sample *t* test comparing NC-low and NC-high groups with autopsy-confirmed AD subjects.
[a]Data from Shaw et al.[15]
CSF = cerebrospinal fluid; NC = normal control; AD = Alzheimer disease; PM = postmortem.

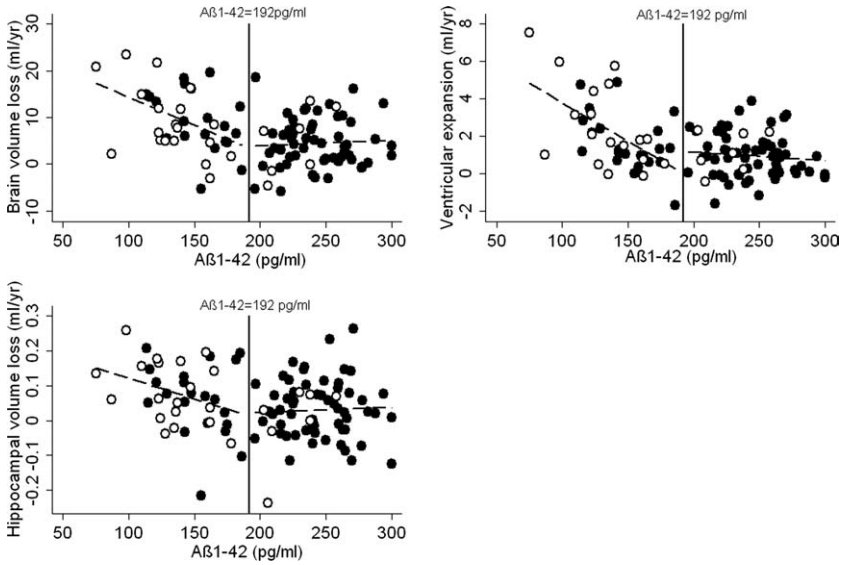

FIGURE: Plots of baseline cerebrospinal fluid Aβ1-42 versus annualized 1-year brain volume change, ventricular change, and hippocampal change. Open circles represent *APOE4*-positive individuals; closed circles represent *APOE4*-negative individuals. Regression slopes are presented separately for normal control (NC)-low (≤192pg/ml) and NC-high (>192pg/ml); for all 3 plots, significant differences in slopes between NC-low and NC-high were found. Significant relationships between volume change for all 3 measures and baseline Aβ1-42 ($p < 0.001$) were found only in the NC-low group.

(187pg/ml). The 3 individuals with clearly low Aβ1-42 also had p-tau and p-tau/Aβ1-42 ratios within the AD range. One also had tau within the AD range and converted to AD at month 36. The individual with borderline Aβ1-42 had p-tau and tau within the control range, and converted to MCI at month 36; however, worsening diabetes, gait disturbance, and a new brainstem vascular lesion (apparent on MRI) were also reported. The 2 NC-high group converters all had CSF values within the control range. Of note, 1 was reported to have a significant alcohol problem, and the other had undergone ventriculoperitoneal shunting during the study.

Discussion

The results of this study suggest that a very significant number of cognitively normal adults ~75 years of age have a CSF profile consistent with AD. Evidence from amyloid imaging and CSF studies in this and other cohorts have suggested that Aβ deposition is present in ~1/3 of individuals in this age group,[23,24] and particularly in *APOE4* carriers.[25] Although current AD models suggest that Aβ deposition is a very early pathological feature of AD,[4,26] what has been less clear is whether healthy individuals with evidence of Aβ pathology are inevitably destined to develop AD, and if so, over what timescale. In this study we show that, as well as having higher rates of *APOE4* positivity, the control group with CSF Aβ1-42 levels within the AD range had significantly higher rates of whole brain and hippocampal atrophy and ventricular expansion over the following year compared to those with higher CSF Aβ1-42 levels. Excess cortical atrophy consistent with neurodegeneration is strongly associated with the development of dementia[27] and occurs prior to the onset of symptoms in both familial[28] and sporadic AD.[29] Our data are therefore consistent with the hypothesis that cognitively normal individuals with low CSF Aβ1-42 may not only be at higher risk of developing AD, but may already be some way down the pathogenic pathway. The proportion of individuals falling within the NC-low group is broadly in line with epidemiological predictions of the proportions of the population that will develop AD.[1] If replicated in further studies, these findings have significant implications for identification of groups at risk for AD, and for the design of presymptomatic drug prevention studies.

We found no evidence for baseline differences in MMSE, ADAS-Cog, category fluency, or a stringent test of recall memory (AVLT) between the NC-low and NC-high groups. The only statistically significant difference was on Trails B, a demanding set-shifting task with a timed component, on which NC-low subjects were significantly slower. This suggests that in the absence of memory deficits, subtle cognitive slowing or hesitancy may be a feature of incipient cognitive impairment. We found significant linear associations between performance on Trails B and CSF tau and p-tau. Given that the majority of patients with low Aβ1-42 also had elevated tau/p-tau, further studies are required to assess the timing of decline in performance on Trails B in relation to the changing CSF profile during very early AD.

TABLE 3: Baseline Demographics, *APOE* Status, CSF Profiles, Brain Volumes, and 1-Year Rates of Atrophy in Subjects Converting to MCI during a Maximum of 36 Months of Follow-up

Characteristic	Cutoff[15]	Converters					
		1	2[a]	3	4[b]	5	6[c]
MMSE		30	29	29	28	28	29
ADAS-Cog		10.3	13.7	18.3	20	16	11.7
AVLT-delayed recall		5	6	8	4	6	8
Trails test (A)		36	34	27	46	32	35
Trails test (B)		129	86	83	128	61	103
Category fluency (vegetables)		8	16	14	13	10	7
Category fluency (animals)		12	27	19	11	7	21
APOE genotype		3,4	3,4	3,4	3,3	3,4	3,3
Baseline Aβ1–42, pg/ml	<192pg/ml	159[d]	238	123[d]	235	98[d]	186[d]
Baseline tau, pg/ml	>93pg/ml	121[d]	79	73	42	34	52
Baseline p-tau, pg/ml	>23pg/ml	47[d]	19	35[d]	13	81[d]	15
Baseline tau/Aβ1–42 ratio	>0.39pg/ml	0.76[d]	0.33	0.59[d]	0.18	0.35	0.28
Baseline p-tau/Aβ1–42 ratio	>0.1pg/ml	0.30[d]	0.08	0.28[d]	0.05	0.83[d]	0.08
Baseline brain volume, ml		1019	927	993	1097	1120	1134
Baseline ventricular volume, ml		37.0	6.8	38.6	76.0	42.8	45.5
Baseline hippocampal volume, ml		4.1	4.2	5.0	5.3	5.4	5.7
Whole brain atrophy rate, ml/yr		−0.1	−0.1	6.6	5.4	23.4	−1.3
Ventricular expansion rate, ml/yr		1.8	0.2	2.2	−0.1	6.0	−1.7
Hippocampal atrophy rate, ml/yr		0.196	−0.001	0.164	−0.30	0.260	−0.103
Conversion status, months		MCI 6, AD 36	MCI 24	MCI 24	MCI 24	MCI 24	MCI 36

[a]Alcohol history recorded; patient seeking treatment at 36-month visit.
[b]Patient shunted for hydrocephalus between month 24 and month 36.
[c]Worsening diabetes, gait disturbance, and brainstem vascular disease at month 36.
[d]CSF values falling within the AD cutoffs as determined by Shaw et al.[15]
CSF = cerebrospinal fluid; MCI = mild cognitive impairment; MMSE = Mini Mental State Examination; ADAS-Cog = Alzheimer's Disease Assessment Scale, Cognitive Subscale; AVLT = Auditory Verbal Learning Test; KN-BSI = whole brain boundary shift integral; VBSI = ventricle boundary shift integral; HBSI = HMAPS boundary shift integral; AD = Alzheimer disease.

Significant differences between groups were seen in the proportions of each group falling within the autopsy-confirmed AD range for CSF p-tau, with directionally similar results for CSF tau.[15] Overall, the NC-low group fell between the AD and NC-high group for all CSF measures, again suggesting that these individuals may be in an intermediate stage between healthy ageing and clinical AD.

APOE4 positivity is a well-established risk factor for AD and, similar to De Meyer et al,[24] we found NC-low individuals to have ~5-fold increased chance of being *APOE4* positive compared to the NC-high group. We found no evidence for differences between the groups in *CLU*, *CR1,* or *PICALM* genotype, although power to detect such changes is very low given the small sample size, and in this context it is perhaps notable that the odds ratios are broadly in line with previous genome-wide association studies.[30] There were significant differences in the distribution of the *TOMM40* genotype between NC-low and NC-high individuals. *TOMM40* is associated with risk of AD and lower age at onset.[17] Potkin et al, using genome-wide case/control methodology in the ADNI dataset, determined that the minor allele frequency was ~30% in AD and ~15% in controls.[17] We found the minor allele frequency to be 22.5% in the

NC-low group and 9.2% in the NC-high-group, consistent with the hypothesis that this "control" group comprises patients with very early AD and those undergoing healthy aging. *APOE4* positivity was strongly associated with possession of 1 or more *TOMM40* risk alleles, which could reflect that the 2 genes are in linkage disequilibrium.[31] We found no evidence of an independent effect of *TOMM40* after adjusting for *APOE4* positivity, although power to detect this was reduced by the strong association between *APOE4* positivity and possession of 1 or more *TOMM40* risk alleles.

There is conflicting evidence for a relationship between measures of amyloid burden and brain volume in healthy controls, with 1 study reporting smaller whole brain volumes in individuals with lower CSF Aβ1-42 suggestive of presymptomatic atrophy,[32] whereas another showed larger temporal gray matter volumes in Pittsburgh compound B-PET–positive normal controls in keeping either with brain reserve, or excess amyloid deposition.[33] We found no statistically significant differences in baseline ventricular or hippocampal volumes between the 2 groups, although there was a trend for those in the NC-low group to have larger baseline brain volumes. However, rates of whole brain and hippocampal atrophy and ventricular expansion in the NC-low group were all approximately double those of the NC-high group over the following year. Expressed as a percentage of baseline brain volume, whole brain atrophy rates in the NC-high group were ~0.4%/yr, which is in keeping with previous longitudinal studies of normal ageing.[10] We have previously shown using identical methodology in the ADNI cohort ~1.2%/yr whole brain loss in MCI and ~1.5%/yr in AD.[20] The mean cerebral atrophy rate in the NC-low group (~0.85%/yr) was intermediate between the NC-high group and MCI. We found evidence of relationships between increasing atrophy rate and decreasing CSF Aβ1-42 in the NC-low group, but no evidence of any associations in the NC-high group. A previous study has shown an association between lower CSF Aβ1-42 and ventricular expansion rate in ADNI controls.[34] Here we confirm this relationship, but show that it is driven predominantly by an association in the NC-low group, supporting a link between these markers of AD pathology and evolving neurodegeneration.

The significant excess atrophy in the NC-low group could potentially be harnessed for disease prevention studies; our estimated sample sizes using whole brain atrophy are within the scope of phase 3 studies. Although prevention studies are very likely to be carried out over much longer periods, these data may provide a means of exploring disease modification over shorter intervals, although the confidence intervals of these estimates should be noted, and these findings require replication in independent datasets before this approach can be recommended. Further studies are also required to assess whether as would be predicted, atrophy measured over longer time periods improves power. It will also be of particular interest to see whether amyloid PET positivity, which has been reported in ~25–33% of elderly controls, and particularly *APOE4*-positive individuals,[25,35] and which predicts conversion to AD,[13] may provide a noninvasive means of identifying individuals for presymptomatic trials using atrophy or other biomarkers as outcome measures.

Despite supportive evidence from other CSF markers, increased brain atrophy rates and genetics, the major limitation of the study is that we cannot yet confirm which individuals will convert to AD, and when. It is however notable that of the 6 individuals changing diagnosis during the study, 3 were clearly classified within the prodromal AD group, including 1 individual who has subsequently converted to AD. Regarding the others with non-AD CSF profiles, alternative explanations for their cognitive impairment could be suggested. These findings also raise fundamental issues relating to the positive and negative predictive value of CSF biomarkers, and whether, as has been proposed, such biomarkers should now be included in diagnostic criteria for AD.[36] It is therefore critically important to have longer term follow-up and ultimately autopsy confirmation of diagnosis in subjects in this and other longitudinal biomarker studies. Other potential limitations of the study include the relatively high percentage of amyloid-positive normal controls, which may or may not reflect the true population prevalence of individuals with significant amyloid pathology in this age range. There are also a number of issues relating to the reproducibility, reliability, and reporting of CSF Aβ, tau, and p-tau levels, which need to be standardized to allow for cross-study comparisons.

Whether excess rates of brain atrophy in apparently cognitively normal aged patients with CSF profiles suggestive of AD inevitably lead to cognitive impairment, and if so over what time frame, needs to be established. If this proves to be the case, the results we present have significant implications for very early intervention, demonstrating that biomarkers may be used not only to identify AD pathology in asymptomatic individuals, but also to demonstrate and quantify presymptomatic neurodegeneration. This suggests that disease-modifying trials in asymptomatic individuals with the aim of preventing progression to cognitive impairment and dementia may be feasible.

Acknowledgments

This work was undertaken at University College London Hospitals/University College London, which received a

proportion of funding from the Department of Health's National Institute for Health Research Biomedical Research Centres funding scheme. The Dementia Research Centre is an Alzheimer's Research Trust Coordinating Centre and has also received equipment funded by the Alzheimer's Research Trust.

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, AstraZeneca AB, Bayer Schering Pharma AG, Bristol-Myers Squibb, Eisai Global Clinical Development, Elan Corporation, Genentech, GE Healthcare, GlaxoSmithKline, Innogenetics, Johnson and Johnson, Eli Lilly and Co., Medpace, Merck and Co., Novartis AG, Pfizer, F. Hoffman-La Roche, Schering-Plough, and Synarc, as well as nonprofit partners the Alzheimer's Association and Alzheimer's Drug Discovery Foundation, with participation from the US Food and Drug Administration. Private sector contributions to ADNI are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The guarantee 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 Neuro Imaging at the University of California, Los Angeles. This research was also supported by National Institutes of Health grants P30 AG010129 and K01 AG030514, and by the Dana Foundation

J.M.S. is a Higher Education Funding Council for England Clinical Senior Lecturer. J.W.B.'s contribution to the article was as part of a grant in which the London School of Hygiene and Tropical Medicine provides ongoing statistical support to the Dementia Research Centre, Institute of Neurology, University College London. N.C.F. is a Medical Research Council Senior Clinical Scientist. J.B. is an Alzheimer's Research Trust Research Fellow.

We thank Drs S. Crutch and E. Warrington for neuropsychology advice, and Drs I. Malone, M. Clarkson, G. Ridgway, and K. Leung for help with data processing and image analysis.

Potential Conflicts of Interest

J.M.S.: employment, University College London; Grants/grants pending, Alzheimer's Research Trust. N.C.F.: board membership, Alzheimer's Research Forum, Alzheimer's Disease and Associated Disorders, Alzheimer's Society, Alzheimer's Research Trust, Anonymous Foundation Charitable Gift Fund, Banner Health, BioMed Central Alzheimer's Research and Therapy, Neurodegenerative Diseases, University College London, University College London Trust; consultancy, Abbott Laboratories, Lundbeck, GE Healthcare, Elan Pharmaceuticals, Eli Lilly, Eisai, Wyeth Pharmaceuticals, Sanofi-Aventis; employment, University College London; grants/grants pending, Medical Research Council, National Institutes of Health, Alzheimer's Research Trust Grant, Engineering and Physical Sciences Research Council Grant, Anonymous Charitable Foundation; honoraria, GE Healthcare, Movement Disorders Society, Elan/Wyeth Pharmaceuticals, American Neurological Association; patents, QA Box; royalties, license for MIDAS (image analysis program); trial support for contracted image analysis research, GlaxoSmith Kline, Elan Pharmaceuticals, Wyeth Pharmaceuticals, Lundbeck, Sanofi-Aventis, IXICO, Pfizer, Janssen Pharmaceuticals. J.B.: employment, Alzheimer Research Trust; grants/grants pending, Wellcome Trust; travel expenses, Alzheimer's Association.

References

1. Ferri CP, Prince M, Brayne C, et al. Global prevalence of dementia: a Delphi consensus study. Lancet 2005;366:2112–2117.
2. Alzheimer's Association. Changing the trajectory of Alzheimer's disease: a national imperative. Available at: http://www.alz.org/documents_custom/trajectory.pdf Accessed August 20th, 2010.
3. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. Acta Neuropathologica 1991;82:239–259.
4. Jack CR Jr, Knopman DS, Jagust WJ, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol 2010;9:119–128.
5. Petersen RC, Stevens JC, Ganguli M, et al. Practice parameter: early detection of dementia: mild cognitive impairment (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 2001;56:1133–1142.
6. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939–944.
7. Vemuri P, Wiste HJ, Weigand SD, et al. Serial MRI and CSF biomarkers in normal aging, MCI, and AD. Neurology 2010;75:143–151.
8. Klunk WE, Engler H, Nordberg A, et al. Imaging brain amyloid in Alzheimer's disease with Pittsburgh compound-B. Ann Neurol 2004;55:306–319.
9. Chen K, Langbaum JB, Fleisher AS, et al. Twelve-month metabolic declines in probable Alzheimer's disease and amnestic mild cognitive impairment assessed using an empirically pre-defined statistical region-of-interest: findings from the Alzheimer's Disease Neuroimaging Initiative. Neuroimage 2010;51:654–664.
10. Fox NC, Schott JM. Imaging cerebral atrophy: normal ageing to Alzheimer's disease. Lancet 2004;363:392–394.
11. Cummings JL. Integrating ADNI results into Alzheimer's disease drug development programs. Neurobiol Aging 2010;31:1481–1492.

12. Reiman EM, Langbaum JB, Tariot PN. Alzheimer's prevention initiative: a proposal to evaluate presymptomatic treatments as quickly as possible. Biomark Med 2010;4:3–14.
13. Morris JC, Roe CM, Grant EA, et al. Pittsburgh compound B imaging and prediction of progression from cognitive normality to symptomatic Alzheimer disease. Arch Neurol 2009;66:1469–1475.
14. Li G, Sokal I, Quinn JF, et al. CSF tau/Abeta42 ratio for increased risk of mild cognitive impairment: a follow-up study. Neurology 2007;69:631–639.
15. Shaw LM, Vanderstichele H, Knapik-Czajka M, et al. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. Ann Neurol 2009;65:403–413.
16. Saykin AJ, Shen L, Foroud TM, et al. Alzheimer's Disease Neuroimaging Initiative biomarkers as quantitative phenotypes: genetics core aims, progress, and plans. Alzheimers Dement 2010;6:265–273.
17. Potkin SG, Guffanti G, Lakatos A, et al. Hippocampal atrophy as a quantitative trait in a genome-wide association study identifying novel susceptibility genes for Alzheimer's disease. PLoS One 2009;4:e6501.
18. Harold D, Abraham R, Hollingworth P, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet 2009;41:1088–1093.
19. Jack CR Jr, Bernstein MA, Fox NC, et al. The Alzheimer's Disease Neuroimaging Initiative (ADNI): MRI methods. J Magn Reson Imaging 2008;27:685–691.
20. Schott JM, Bartlett JW, Barnes J, et al. Reduced sample sizes for atrophy outcomes in Alzheimer's disease trials: baseline adjustment. Neurobiol Aging 2010;31:1452–1462.
21. Leung KK, Clarkson MJ, Bartlett JW, et al. Robust atrophy rate measurement in Alzheimer's disease using multi-site serial MRI: tissue-specific intensity normalization and parameter selection. Neuroimage 2010;50:516–523.
22. Leung KK, Barnes J, Ridgway GR, et al. Automated cross-sectional and longitudinal hippocampal volume measurement in mild cognitive impairment and Alzheimer's disease. Neuroimage 2010;51:1345–1359.
23. Visser PJ, Verhey F, Knol DL, et al. Prevalence and prognostic value of CSF markers of Alzheimer's disease pathology in patients with subjective cognitive impairment or mild cognitive impairment in the DESCRIPA study: a prospective cohort study. Lancet Neurol 2009;8:619–627.
24. De Meyer G, Shapiro F, Vanderstichele H, et al. Diagnosis-independent Alzheimer disease biomarker signature in cognitively normal elderly people. Arch Neurol 2010;67:949–956.
25. Morris JC, Roe CM, Xiong C, et al. APOE predicts amyloid-beta but not tau Alzheimer pathology in cognitively normal aging. Ann Neurol 2010;67:122–131.
26. Perrin RJ, Fagan AM, Holtzman DM. Multimodal techniques for diagnosis and prognosis of Alzheimer's disease. Nature 2009;461:916–922.
27. Savva GM, Wharton SB, Ince PG, et al. Age, neuropathology, and dementia. N Engl J Med 2009;360:2302–2309.
28. Schott JM, Fox NC, Frost C, et al. Assessing the onset of structural change in familial Alzheimer's disease. Ann Neurol 2003;53:181–188.
29. Rusinek H, De Santi S, Frid D, et al. Regional brain atrophy rate predicts future cognitive decline: 6-year longitudinal MR imaging study of normal aging. Radiology 2003;229:691–696.
30. Bertram L, McQueen MB, Mullin K, et al. Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. Nat Genet 2007;39:17–23.
31. Hong MG, Alexeyenko A, Lambert JC, et al. Genome-wide pathway analysis implicates intracellular transmembrane protein transport in Alzheimer disease. J Hum Genet 2010;55:707–709.
32. Fagan AM, Head D, Shah AR, et al. Decreased cerebrospinal fluid Abeta(42) correlates with brain atrophy in cognitively normal elderly. Ann Neurol 2009;65:176–183.
33. Chetelat G, Villemagne VL, Pike KE, et al. Larger temporal volume in elderly with high versus low beta-amyloid deposition. Brain 2010;133:3349–3358.
34. Tosun D, Schuff N, Truran-Sacrey D, et al. Relations between brain tissue loss, CSF biomarkers, and the ApoE genetic profile: a longitudinal MRI study. Neurobiol Aging 2010;31:1340–1354.
35. Rowe CC, Ellis KA, Rimajova M, et al. Amyloid imaging results from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging. Neurobiol Aging 2010;31:1275–1283.
36. Dubois B, Feldman HH, Jacova C, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet Neurol 2007;6:734–746.