# BRAIN
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# Cognitive reserve and Alzheimer's disease biomarkers are independent determinants of cognition

Prashanthi Vemuri,[1] Stephen D. Weigand,[2] Scott A. Przybelski,[2] David S. Knopman,[3] Glenn E. Smith,[4] John Q. Trojanowski,[5] Leslie M. Shaw,[5] Charlie S. Decarli,[6] Owen Carmichael,[6] Matt A. Bernstein,[1] Paul S. Aisen,[7] Michael Weiner,[8] Ronald C. Petersen[3] and Clifford R. Jack Jr[1] on behalf of the Alzheimer's Disease Neuroimaging Initiative

1 Department of Radiology, Mayo Clinic and Foundation, Rochester, MN 55905, USA
2 Department of Health Sciences Research, Mayo Clinic and Foundation, Rochester, MN 55905, USA
3 Department of Neurology, Mayo Clinic and Foundation, Rochester, MN 55905, USA
4 Department of Psychology, Mayo Clinic and Foundation, Rochester, MN 55905, USA
5 Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA
6 Department of Neurology and Centre of Neuroscience, University of California at Davis, Davis, CA 95618, USA
7 Department of Neurosciences, University of California at San Diego, San Diego, CA 92093, USA
8 Veterans Affairs and University of California, San Francisco, CA 94121, USA

Correspondence to: Prashanthi Vemuri, PhD,
Mayo Clinic and Foundation,
200 First Street SW,
Rochester, MN 55905, USA
E-mail: vemuri.prashanthi@mayo.edu

The objective of this study was to investigate how a measure of educational and occupational attainment, a component of cognitive reserve, modifies the relationship between biomarkers of pathology and cognition in Alzheimer's disease. The biomarkers evaluated quantified neurodegeneration via atrophy on magnetic resonance images, neuronal injury via cerebral spinal fluid t-tau, brain amyloid-β load via cerebral spinal fluid amyloid-$β_{1-42}$ and vascular disease via white matter hyperintensities on $T_2$/proton density magnetic resonance images. We included 109 cognitively normal subjects, 192 amnestic patients with mild cognitive impairment and 98 patients with Alzheimer's disease, from the Alzheimer's Disease Neuroimaging Initiative study, who had undergone baseline lumbar puncture and magnetic resonance imaging. We combined patients with mild cognitive impairment and Alzheimer's disease in a group labelled 'cognitively impaired' subjects. Structural Abnormality Index scores, which reflect the degree of Alzheimer's disease-like anatomic features on magnetic resonance images, were computed for each subject. We assessed Alzheimer's Disease Assessment Scale (cognitive behaviour section) and mini-mental state examination scores as measures of general cognition and Auditory–Verbal Learning Test delayed recall, Boston naming and Trails B scores as measures of specific domains in both groups of subjects. The number of errors on the American National Adult Reading Test was used as a measure of environmental enrichment provided by educational and occupational attainment, a component of cognitive reserve. We found that in cognitively normal subjects, none of the biomarkers correlated with the measures of cognition, whereas American National Adult Reading Test scores were significantly correlated with Boston naming and mini-mental state examination results. In cognitively impaired subjects, the American National Adult Reading Test and all

biomarkers of neuronal pathology and amyloid load were independently correlated with all cognitive measures. Exceptions to this general conclusion were absence of correlation between cerebral spinal fluid amyloid-$\beta_{1-42}$ and Boston naming and Trails B. In contrast, white matter hyperintensities were only correlated with Boston naming and Trails B results in the cognitively impaired. When all subjects were included in a flexible ordinal regression model that allowed for non-linear effects and inter-actions, we found that the American National Adult Reading Test had an independent additive association such that better performance was associated with better cognitive performance across the biomarker distribution. Our main conclusions included: (i) that in cognitively normal subjects, the variability in cognitive performance is explained partly by the American National Adult Reading Test and not by biomarkers of Alzheimer's disease pathology; (ii) in cognitively impaired subjects, the American National Adult Reading Test, biomarkers of neuronal pathology (structural magnetic resonance imaging and cerebral spinal fluid t-tau) and amyloid load (cerebral spinal fluid amyloid-$\beta_{1-42}$) all independently explain variability in general cognitive performance; and (iii) that the association between cognition and the American National Adult Reading Test was found to be additive rather than to interact with biomarkers of Alzheimer's disease pathology.

# Introduction

Subjects with Alzheimer's disease at autopsy typically were de-mented in life. However, careful autopsy studies consistently reveal an important clinical–pathological discordance. Up to 30% of subjects who were cognitively normal in life are found at aut-opsy to have a pathological profile, typically dominated by amyl-oid plaques, consistent with a diagnosis of Alzheimer's disease (Katzman *et al.*, 1988; Crystal *et al.*, 1993; Hulette *et al.*, 1998; Price and Morris, 1999; Schmitt *et al.*, 2000; Morris and Price, 2001; Riley *et al.*, 2002; Knopman *et al.*, 2003). Understanding this disassociation between cognition and pathology is important for the success of preventive strategies targeted at neuropathology in the non-demented elderly as well as relevant for improving our understanding of the disease. The concept of cognitive reserve (Stern, 2009) is increasingly employed to explain this disassoci-ation. This theory posits that subjects with higher cognitive reserve have a greater capacity to cope with pathological insults than those with low cognitive reserve, and these individual differences in reserve mechanisms help explain why cognitive decline may be initiated at different times in relation to the onset of pathology. This concept can also be expressed as the difference between the predicted and the observed cognitive performance of an individual for a given level of brain pathology (Reed *et al.*, 2010). Figure 1 illustrates the question we are addressing—how does cognitive reserve modify the impact of pathology on cognition? In this art-icle, we investigate one aspect of cognitive reserve as measured by the number of errors on the American National Adult Reading Test (AMNART), an indicator of pre-morbid verbal intelligence. While AMNART does not capture all the components that contribute to cognitive reserve, it is a measure of the level of environmental enrichment provided by educational and occupational attainment and has been shown to correlate with IQ (Ryan and Paolo, 1992).

Dementia is a multi-factorial disease wherein cumulative patho-logical brain insults (usually more than one pathology) result in progressive cognitive decline, which ultimately leads to dementia.

Amyloid plaques and neurofibrillary tangles are the well-established pathological hallmarks of Alzheimer's disease. The other main pathological processes in Alzheimer's disease are inflammation and neurodegeneration, which is due to the loss of neurons, synapses and dendritic de-arborization that occurs on a microscopic level. In this article, we restrict ourselves to the pri-mary pathologies of Alzheimer's disease as well as to ischaemic cerebrovascular disease since it is the most common secondary pathological process seen among demented subjects with Alzheimer's disease pathology at autopsy (Snowdon *et al.*, 1997; White *et al.*, 2005; Schneider *et al.*, 2009). We use MRI and CSF biomarkers to quantify each of these pathological changes. Low CSF amyloid-$\beta_{1-42}$ reflects deposition of amyloid-$\beta$ in plaques (Strozyk *et al.*, 2003; Tapiola *et al.*, 2009). High CSF t-tau levels reflect active axonal and neuronal damage (Blennow *et al.*, 1995). Atrophy seen on MRI is the macroscopic result of loss of neurons, synapses and dendritic arborization (Bobinski *et al.*, 2000). We use the Structural Abnormality Index score as an indicator of the se-verity of the Alzheimer's disease-like pattern of atrophy on struc-tural MRI. Structural Abnormality Index scores were developed to condense the severity and location of Alzheimer's disease-related atrophy on the 3D MRI scan into a single number (Vemuri *et al.*, 2008). White matter hyperintensity (or leukoaraiosis) on fluid-attenuation inversion recovery MRI in elderly subjects is sus-pected to be a direct manifestation of microvascular ischaemic injury. We use white matter hyperintensity load as an indicator of severity of small vessel disease.

Several recent studies have used biomarkers to understand the effect of cognitive reserve on the relationship between pathology and cognition (Stern, 2009). These studies have examined the effect of cognitive reserve on cross-sectional measures of amyloid load (Kemppainen *et al.*, 2008; Roe *et al.*, 2008a; Rentz *et al.*, 2010; Dumurgier *et al.*, 2010), neurodegeneration (Querbes *et al.*, 2009; Piras *et al.*, 2010), fibre tract integrity (Teipel *et al.*, 2009), white matter hyperintensity (Brickman *et al.*, 2009b), cerebral me-tabolism (Alexander *et al.*, 1997; Scarmeas *et al.*, 2003; Perneczky
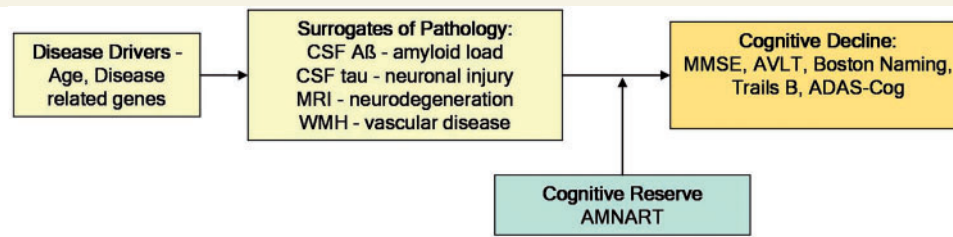
**Figure 1** Illustration of where cognitive reserve acts along the pathology–cognitive decline cascade. Aβ = amyloid-β; AVLT = Auditory–Verbal Learning Test; WMH = white matter hyperintensity volume.

*et al.*, 2007; Garibotto *et al.*, 2008; Hanyu *et al.*, 2008; Cohen *et al.*, 2009) and perfusion (Liao *et al.*, 2005) independently. Autopsy studies have shown the association of plaques and tangles with cognitive reserve (Bennett *et al.*, 2003; Boyle *et al.*, 2008; Koepsell *et al.*, 2008; Roe *et al.*, 2008*b*). However, we know of no *in vivo* studies that have investigated the effect of cognitive reserve on all the primary pathologies seen in Alzheimer's disease including neurodegeneration and the most common secondary pathology (i.e. vascular disease) in the same cohort of subjects that spans the cognitive spectrum from normal to mild Alzheimer's disease.

The main aim of our article is to investigate how the component of cognitive reserve measured by AMNART modifies the relationship between biomarkers of pathology and cognition in Alzheimer's disease by answering these two questions: (i) does AMNART explain variability in cognitive measures, even after adjusting for biomarkers of pathology within cognitively normal and impaired subjects?; and (ii) does AMNART have an additive association with cognition or does it interact with biomarkers of pathology?

# Materials and methods

The data used in this study are from the Alzheimer's Disease Neuroimaging Initiative, a longitudinal multicentre observational study of elderly individuals with normal cognition, amnestic mild cognitive impairment and Alzheimer's disease collected from 59 participating institutes (Jack *et al.*, 2008). Written informed consent was obtained for participation in these studies, as approved by the Institutional Review Board at each of the participating centres. The details of the Alzheimer's Disease Neuroimaging Initiative can be found at http://www.ADNI-info.org.

## Clinical and cognitive assessment

The total sample in this article consists of 399 subjects (109 cognitively normal, 192 amnestic mild cognitive impairment, 98 Alzheimer's disease) who had CSF biomarker data at baseline (CSF was obtained at baseline in ~51% of the Alzheimer's Disease Neuroimaging Initiative cohort). Baseline clinical diagnosis and cognitive assessments of all three clinical groups and clinical/cognitive assessment scores were considered in this study. AMNART was obtained in each of the individuals (Ryan and Paolo, 1992) and the number of errors on AMNART was used as a measure of cognitive reserve in this study. The AMNART evaluates pre-morbid verbal IQ in individuals and is determined by the number of errors made on a pronunciation list of 50 irregularly spelled words such as 'ache', 'debt' and 'bouquet'. Alternative proxies of cognitive reserve exist, such as education, but we chose AMNART since it is more sensitive and shows greater variation than education (Rentz *et al.*, 2010). While the Alzheimer's Disease Neuroimaging Initiative generally classifies subjects as cognitively normal, having mild cognitive impairment, or Alzheimer's disease, for this study, we combined subjects who had mild cognitive impairment and Alzheimer's disease at baseline into a single impaired group since the distribution of AMNART was very similar in amnestic-mild cognitive impairment and Alzheimer's disease. In this way, the impaired group represents a broad spectrum of cognitive impairment and also shows a greater dynamic range of values on standard cognitive tests.

We used Alzheimer's Disease Assessment Scale—Cognitive Behaviour Section (ADAS-Cog; Rosen *et al.*, 1984) and Mini-Mental State Examination (MMSE; Folstein *et al.*, 1975) as overall indices of general cognitive performance. In this study, we used the modified ADAS-Cog score (ADAS-Cog-13) from the Alzheimer's Disease Neuroimaging Initiative, which has two additional components (delayed recall task and a number cancellation task). Additionally, we used domain-specific scores—Auditory–Verbal Learning Test-delayed recall (Rey, 1964) as a measure of memory encoding, Boston naming (Kaplan *et al.*, 1983) to test language functioning and Trails B (US War Department, 1944) as a measure of executive function. We analysed all tests in all subjects.

## Cerebral spinal fluid methods and processing

CSF was collected at each site, transferred into polypropylene transfer tubes, frozen on dry ice within 1 h after collection and shipped overnight to the Alzheimer's Disease Neuroimaging Initiative Biomarker Core laboratory at the University of Pennsylvania Medical Centre on dry ice. When samples were received in the Laboratory, they were thawed and aliquots were stored in bar coded polypropylene vials at −80°C. A standardized protocol was implemented to quantify biomarker concentrations in each of the CSF Alzheimer's Disease Neuroimaging Initiative baseline aliquots using a multiplex xMAP Luminex platform (Luminex Corp) with Innogenetics (INNO-BIA AlzBio3, for research use only reagents) immunoassay kit-based reagents, a protocol which has been tested and validated (Vanderstichele, 2008; Shaw *et al.*, 2009). Quality control values obtained during the analyses of Alzheimer's Disease Neuroimaging Initiative baseline CSF aliquots were: inter-day reproducibilities (%CV) for an Alzheimer's disease CSF pool and a routine clinic patient CSF pool of 4.5% and 6.4% for t-tau and 3.3% and 6.9%

for amyloid-$\beta_{1-42}$; $r^2$ values for comparison of retested samples were 0.98 and 0.90. Baseline CSF values were considered in this study.

## Magnetic resonance imaging methods and preprocessing

The 1.5T MRI scans from the Alzheimer's Disease Neuroimaging Initiative were used for this study. The nominal parameters of the morphometric $T_1$-weighted magnetization prepared rapid acquisition gradient echo imaging sequence and $T_2$-weighted MRI scans can be found in Jack *et al.* (2008). The $T_1$ MRI images are corrected for gradient non-linearity and intensity inhomogeneity (Jack *et al.*, 2008) using a centralized MRI-processing pipeline at the Mayo Clinic, Rochester. Structural Abnormality Index-scores were estimated on these preprocessed images and are described in the online Supplementary Material. The $T_2$/PD and $T_1$-weighted images were used to estimate the white matter hyperintensity load based on the method presented in (Schwarz *et al.*, 2009) (http://www.ncbi.nlm.nih .gov/pmc/articles/PMC2864489/).

## Statistical analysis

### Correlations between American National Adult Reading Test, biomarkers and cognition

We estimated the association between variables using partial Spearman rank-order correlation, which we denote by 'partial $r_s$'. All correlations adjust for, or partial out, the effects of age and gender. We also report partial correlations after further adjusting for biomarker or AMNART. Rank-order correlations were used because of skewness and otherwise non-normal distributions resulting from floor or ceiling effects. Partial correlations were calculated using SAS version 9.1.3.

An important goal of this study was to characterize how mean cognition changes with AMNART and biomarker levels and to assess whether the possibly protective effect of cognitive reserve is additive, whereby it is relatively constant across levels of neuropathology, or whether there is an interaction in which the protective effect of cognitive reserve depends on the level of the biomarker as would be the case, for example, if cognitive reserve offered protection only at mild levels of neuropathology. To address these questions, we used proportional odds ordinal logistic regression models (Harrell, 2001) treating the cognitive test as an ordinal response. These models generalize binary logistic regression to the case where there are more than two categories but they have a natural ordering. One interpretation of these models is that they treat the observed score on a cognitive test as a discrete or 'coarsened' estimate of an unobservable, or latent, cognitive quantity that falls along a true continuum that is assumed to have an underlying continuous logistic distribution. As binary logistic regression can provide the estimated probability of an event or non-event as a function of covariates, proportional odds logistic regression models can be used to estimate the probability of each level of the ordinal response, e.g. MMSE = 30, 29, 28, etc. We then calculate the estimated mean value for the cognitive test as a function of covariates by summing up the scores multiplied by their probabilities (Hannah and Quigley, 1996). For example, if a test took on values 1, 2 or 3, and the estimated probabilities were 0.20, 0.30 and 0.50, the mean would be 1(0.20) + 2(0.30) + 3(0.50) = 2.3. With this approach to estimating the mean in an ordinal logistic regression model, we avoid the

problems of using linear regression when there are strong floor or ceiling effects or a large number of ties.

To reduce skewness, we transformed AMNART by taking the square root and took the base 2 logarithm of amyloid-$\beta_{1-42}$, t-tau, and white matter hyperintensity volume. So as not to assume strictly linear relationships, in all models we fit the biomarker and AMNART predictors as restricted cubic splines with knots at the 10th, 50th and 90th percentiles (Harrell, 2001). To assess the extent to which the relationship between AMNART and cognition varied by pathology level, we included a linear interaction, or product term, whereby the AMNART score was multiplied by the pathology measure (Harrell, 2001). To adjust for possible age effects and sex effects in the MMSE models we included age, sex, an age by sex interaction, and allowed age and sex to interact with the linear components of the biomarker and AMNART terms.

In all, the full models for MMSE had 18 degrees of freedom. While models of this complexity are reasonable given the large sample size, in order to obtain more stable and smoother estimates, we used a ridge-regression penalization approach with the penalty factor chosen to optimize the 'corrected Akaike Information Criterion' (Harrell, 2001). For the MMSE, the penalization reduced the effective degrees of freedom for the amyloid-$\beta_{1-42}$ model to 6.3 degrees of freedom, the Structural Abnormality Index model to 9.2 degrees of freedom, the t-tau model to 7.3 degrees of freedom and the white matter hyperintensity model to 6.6 degrees of freedom. We used large-sample Wald tests on the full model to assess: (i) the interaction between biomarker and AMNART; (ii) the biomarker effect controlling for age, sex and AMNART; and (iii) the AMNART effect controlling for age, sex and biomarker. Ordinal regression modelling was performed using Harrell's Design package and R version 2.8.1 (R Development Core Team, 2008).

To explore the effects of measurement error in AMNART and biomarkers on our ordinal regression model estimates, we performed a sensitivity analysis using the simulation-extrapolation method (Cook and Stefanski, 1994). The idea behind this approach is to add measurement error to the predictors and then re-estimate the regression coefficients. By adding increasing amounts of measurement error, for a given regression coefficient, one can plot the resulting estimate on the *y*-axis and the amount of measurement error on the *x*-axis and then fit a trend line to these observations. One then extrapolates from this trend line the value of the regression coefficient assuming zero measurement error. We performed the simulation-extrapolation method five times by assuming measurement error was such that the intra-class correlation coefficient for AMNART and the biomarkers was 0.50, 0.60, 0.70, 0.80 and 0.90.

# Results

## Patient characteristics

The demographics, clinical summary, biomarker characteristics of cognitively normal, cognitively impaired subjects (amnestic mild cognitive impairment and Alzheimer's disease) are shown in Table 1. Figure 2 shows the distribution of AMNART scores separately for cognitively normal, amnestic mild cognitive impairment and Alzheimer's disease. Based on the Wilcoxon rank-sum/Mann–Whitney U-test, the number of errors was significantly lower for the cognitively normal group than amnestic mild cognitive

**Table 1** Patient characteristics at baseline

| | Cognitively normal (n = 109) | Cognitively impaired (n = 290) |
|---|---|---|
| Gender: female, n (%) | 52 (48) | 105 (36) |
| Apolipoprotein E ε4 carriers, n (%) | 27 (25) | 172 (59) |
| Age, median (IQR), years | 76 (72–78) | 75 (70–80) |
| Education, median (IQR), years | 16 (14–18) | 16 (13–18) |
| Amyloid-$\beta_{1–42}$, median (IQR), pg/ml | 221 (154–248) | 143 (127–169) |
| t-tau, median (IQR), pg/ml | 63 (47–87) | 96 (68–135) |
| Structural Abnormality Index, median (IQR) | −0.9 (−1.5 to −0.4) | 0.2 (−0.5 to 0.9) |
| White matter hyperintensity volume, median (IQR), cm³ | 0.2 (0.1–0.5) | 0.3 (0.1–0.8) |
| AMNART, median (IQR) | 8 (4–15) | 14 (7–21) |
| MMSE, median (IQR) | 29 (29–30) | 26 (24–28) |
| ADAS-Cog, median (IQR) | 9.7 (6.3–13.0) | 21.7 (16.7–27.3) |
| CDR-SB, median (IQR) | 0.0 (0.0–0.0) | 2.0 (1.0–3.5) |
| Auditory–Verbal Learning Test delayed recall, median (IQR) | 7 (5–10) | 1 (0–3) |
| Boston Naming, median (IQR) | 28 (26–30) | 26 (23–28) |

CDR-SB = Clinical Dementia Rating 'sum of boxes'; IQR = interquartile range defined as (25th percentile, 75th percentile).
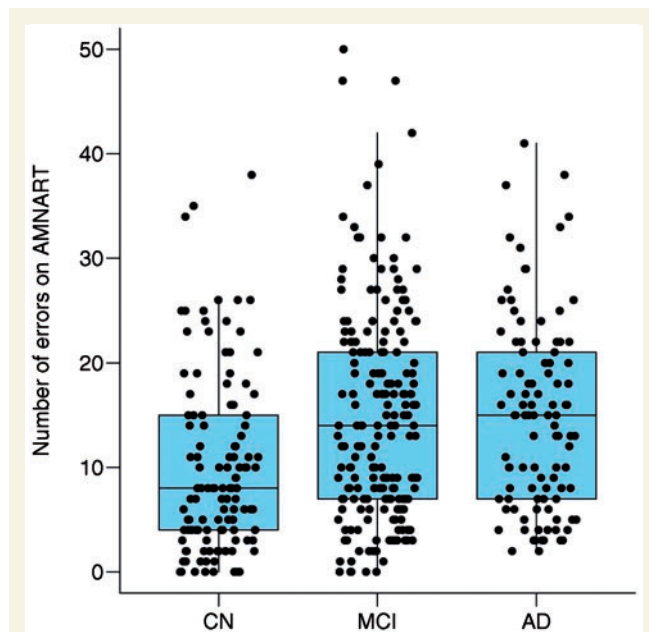


**Figure 2** Box plots of the distribution of AMNART errors by clinical group. The horizontal lines within each box represent the 25, 50 and 75th percentiles. The vertical lines extend out to the furthest point within 1.5 interquartile ranges of the box, where an interquartile range is the 75th minus the 25th percentiles. AD = Alzheimer's disease; CN = cognitively normal; MCI = mild cognitive impairment.

## Correlations between American National Adult Reading Test, biomarkers and cognition

### Cognitively normal subjects

Among cognitively normal subjects, we found that none of the biomarkers (CSF amyloid-$\beta_{1–42}$, CSF t-tau, Structural Abnormality Index or white matter hyperintensity volume) correlated with the measures of cognition we assessed with all correlations <0.17 in absolute value ($P > 0.05$). In contrast AMNART correlated with MMSE (age and gender adjusted partial $r_s = −0.37$, $P < 0.01$) and Boston naming (age and gender adjusted partial $r_s = −0.31$, $P < 0.01$) but did not correlate with ADAS-Cog, Auditory–Verbal Learning Test or Trails B ($P > 0.05$). The magnitude of the correlation between MMSE and AMNART was similar (partial $r_s = −0.37$ to −0.36, $P < 0.01$) after adjusting for each of the CSF and MRI biomarkers individually, as well as when all biomarkers of pathology where combined (partial $r_s = −0.36$, $P < 0.01$). Similarly the correlation between Boston naming and AMNART were similar before and after adjusting for each of the CSF and MRI biomarkers individually as well as when all were combined. These correlations are illustrated in Table 2.

### Cognitively impaired subjects

Among impaired subjects, the biomarkers of Alzheimer's disease (CSF amyloid-$\beta_{1–42}$, CSF t-tau and MRI) correlated with the measures of general cognitive performance (ADAS-Cog, MMSE; age- and gender-adjusted absolute partial $r_s = 0.19–0.51$, $P < 0.01$). These correlations were little changed after further adjusting for AMNART (absolute partial $r_s = 0.17–0.51$, $P < 0.01$). Most of the Alzheimer's disease biomarkers correlated with the domain scores (Auditory–Verbal Learning Test, Boston naming, Trails B). The exception was absence of correlation between CSF amyloid-$\beta_{1–42}$ with Boston naming and Trails B. These correlations were little changed as well after adjusting for AMNART. White matter

impairment (area under receiver operating characteristic curve = 0.64, $P < 0.001$) or Alzheimer's disease (area under receiver operating characteristic curve = 0.67, $P < 0.001$) while amnestic mild cognitive impairment and Alzheimer's disease were not different (area under receiver operating characteristic curve = 0.52, $P = 0.53$).

**Table 2 Partial Spearman rank correlations for the cognitively normal patients**

| Predictor | Adjustment variables[a] | Cognitive measurement | | | | |
|---|---|---|---|---|---|---|
| | | ADAS-Cog | MMSE | Auditory–Verbal Learning Test | Boston Naming | Trails B |
| **Predictor variable** | | | | | | |
| Amyloid-$\beta_{1-42}$ | | −0.14 | −0.14 | 0.04 | 0.12 | −0.11 |
| STAND | | 0.07 | 0.01 | −0.04 | −0.16 | 0.04 |
| t-tau | | 0.06 | 0.05 | −0.01 | −0.03 | 0.14 |
| WMH | | 0.17 | 0.14 | 0.04 | 0.04 | −0.04 |
| AMNART | | 0.16 | −0.37** | −0.05 | −0.31** | 0.07 |
| **AMNART adjusted for biomarkers** | | | | | | |
| AMNART | Amyloid-$\beta_{1-42}$ | 0.17 | −0.36** | −0.06 | −0.32** | 0.08 |
| AMNART | STAND | 0.16 | −0.37** | −0.05 | −0.31** | 0.07 |
| AMNART | t-tau | 0.16 | −0.37** | −0.05 | −0.31** | 0.06 |
| AMNART | WMH | 0.17 | −0.36** | −0.05 | −0.32** | 0.07 |
| AMNART | Amyloid-$\beta_{1-42}$, t-tau, STAND and WMH | 0.18 | −0.36** | −0.06 | −0.34** | 0.07 |
| **Biomarkers adjusted for AMNART** | | | | | | |
| Amyloid-$\beta_{1-42}$ | | −0.15 | −0.13 | 0.05 | 0.15 | −0.11 |
| STAND | | 0.07 | 0.00 | −0.04 | −0.17 | 0.04 |
| t-tau | | 0.05 | 0.06 | −0.00 | −0.02 | 0.14 |
| WMH | | 0.18 | 0.12 | 0.04 | 0.03 | −0.03 |

a Age and gender are included.
STAND = Structural Abnormality Index; WMH = white matter hyperintensity.
*P-value between 0.01 and 0.05.
**P-value < 0.01.

hyperintensity did not correlate with the ADAS-Cog, MMSE or Auditory–Verbal Learning Test, but did correlate with domains measures for language (Boston naming) and executive function (Trails B).

AMNART correlated with ADAS-Cog (age and gender adjusted partial $r_s = 0.14$, $P = 0.02$), MMSE (age and gender adjusted partial $r_s = -0.21$, $P < 0.01$), Boston naming (age and gender adjusted partial $r_s = -0.37$, $P < 0.01$) and Trails B (age and gender adjusted partial $r_s = 0.22$, $P < 0.01$). After further adjusting for each of the CSF and MRI biomarkers, the magnitude of correlation of AMNART with cognitive measures was similar irrespective of the biomarker adjusted for. This correlation was also similar after adjusting for all the biomarkers. These correlations are illustrated in Table 3. As can be observed from the table, the correlation of AMNART with the cognitive measures does not change after adjustment by any of the biomarkers. Also, there was no apparent relationship between AMNART and biomarker among impaired subjects, suggesting AMNART, amyloid-$\beta_{1-42}$, t-tau and Structural Abnormality Index are independent predictors of cognitive performance.

# Effect of American National Adult Reading Test on the relationship between biomarkers and cognition

We illustrate the interrelationship between MMSE, biomarker and AMNART in Fig. 3. In each panel, we show MMSE as a function of the biomarker level and show the estimated mean MMSE for three levels of AMNART. We found that MMSE depended significantly on amyloid-$\beta_{1-42}$ ($P < 0.001$), Structural Abnormality Index

($P < 0.001$) and t-tau ($P < 0.001$) but not white matter hyperintensity volume ($P = 0.88$). In all models, the effect of AMNART on the relationship between MMSE and biomarker level was highly significant ($P < 0.001$). In other words, for a given level of cognition, fewer errors on AMNART, i.e. higher cognitive reserve, is associated with higher levels of t-tau, lower levels of CSF amyloid-$\beta_{1-42}$ and greater cerebral atrophy when compared with subjects with low cognitive reserve.

For log CSF amyloid-$\beta_{1-42}$ (Fig. 3A) there is an upward, but possibly non-linear, trend indicating higher levels of amyloid-$\beta_{1-42}$ are associated with better performance on MMSE. Figure 3B shows a clear, approximately linear, downward trend in MMSE as Structural Abnormality Index increases while for any given level of Structural Abnormality Index worse AMNART results in lower average MMSE. For log t-tau, the association with MMSE appears approximately linear. For log white matter hyperintensity, no significant association with MMSE was observed, while the additive effect of AMNART was pronounced. We found no evidence of an interaction between AMNART and the marker ($P > 0.59$ for each marker) and infer that there is an approximate 'additive' association such that for a given level of the biomarker, better performance on AMNART corresponds with an upward shift in average MMSE. In contrast, an interaction between AMNART and biomarker would have resulted in significantly different slopes for the different AMNART groups in the regression of MMSE on biomarkers (Fig. 3), which was not found.

In our sensitivity analysis evaluating the effects of measurement error in AMNART or the biomarkers, we found that increasing amounts of measurement error tended to attenuate observed associations but that our estimates, as summarized graphically in

**Table 3** Partial Spearman rank correlations for the cognitively impaired patients

| Predictor | Adjustment variables[a] | Cognitive measurement | | | | |
|---|---|---|---|---|---|---|
| | | ADAS-Cog | MMSE | Auditory–Verbal Learning Test | Boston Naming | Trails B |
| **Predictor variable** | | | | | | |
| Amyloid-$\beta_{1-42}$ | | −0.27** | 0.19** | 0.21** | 0.00 | −0.09 |
| STAND | | 0.51** | −0.35** | −0.27** | −0.29** | 0.33** |
| t-tau | | 0.22** | −0.19** | −0.23** | −0.12* | 0.14* |
| WMH | | 0.11 | −0.06 | −0.05 | −0.15* | 0.23** |
| AMNART | | 0.14* | −0.21** | −0.03 | −0.37** | 0.22** |
| **AMNART adjusted for biomarkers** | | | | | | |
| AMNART | Amyloid-$\beta_{1-42}$ | 0.16** | −0.22** | −0.04 | −0.37** | 0.23** |
| AMNART | STAND | 0.14* | −0.20** | −0.02 | −0.37** | 0.22** |
| AMNART | t-tau | 0.14* | −0.20** | −0.02 | −0.37** | 0.22** |
| AMNART | WMH | 0.15* | −0.21** | −0.04 | −0.37** | 0.22** |
| AMNART | Amyloid-$\beta_{1-42}$, t-tau, STAND and WMH | 0.16** | −0.21** | −0.02 | −0.37** | 0.21** |
| **Biomarkers adjusted for AMNART** | | | | | | |
| Amyloid-$\beta_{1-42}$ | | −0.28** | 0.20** | 0.21** | 0.02 | −0.10 |
| STAND | | 0.51** | −0.35** | −0.26** | −0.29** | 0.33** |
| t-tau | | 0.22** | −0.17** | −0.22** | −0.12 | 0.14* |
| WMH | | 0.10 | −0.04 | −0.05 | −0.13* | 0.21** |

a Age and gender are included.
STAND = Structural Abnormality Index; WMH = white matter hyperintensity.
*$P$ value between 0.01 and 0.05.
**$P$ value < 0.01.

Fig. 3, were little changed after correction using the simulation-extrapolation method.

# Discussion

The concept of cognitive reserve is being employed increasingly to explain the difference between observed and expected cognitive performance for a given degree of Alzheimer's disease pathology. We investigated the contribution of cognitive reserve on this disassociation using biomarkers that capture two major aspects of Alzheimer's disease pathology (amyloid and neuronal) as well as vascular pathology, which is very common among the elderly population. The major findings from this study are: (i) that in cognitively normal subjects, the variability in cognition is explained partly by AMNART but not by biomarkers of Alzheimer's disease pathology; (ii) in cognitively impaired subjects, AMNART, biomarkers of neuronal pathology (structural MRI and CSF t-tau) and amyloid load (CSF amyloid-$\beta_{1-42}$) were all independently associated with cognitive and functional performance; and (iii) that the association between cognition and AMNART was found to be additive rather than to depend on biomarkers of Alzheimer's disease pathology such that better performance on AMNART corresponded with better cognition across the Alzheimer's disease continuum.

## Cognitive reserve and cognition

The concept of reserve stemmed from the observation that there is often poor one-to-one correspondence between the presence of pathology at autopsy and cognition in life (Katzman et al., 1988). Cognitive reserve is an inclusive term that has been loosely used to explain the inter-subject variability in cognitive performance in the face of brain pathology. There are several processes that constitute cognitive reserve including genetic and environmental influences, number of neurons and synapses, the sensitivity of neurons and glia to pathology, neuroplasticity etc. Stern (2006) formally categorized cognitive reserve mechanisms into two parts: active and passive. Passive cognitive reserve is created by pre-existing networks that are more efficient or have greater capacity, and thus may be less susceptible to disruption by pathology. Active cognitive reserve is created by alternate networks that compensate for pathological disruption of pre-existing networks. AMNART, which measures pre-morbid verbal intelligence, may possibly capture components from both of these cognitive reserve mechanisms.

In cognitively normal subjects we found that AMNART correlated moderately with Boston naming (partial $r_s = -0.31$, $P < 0.01$) and MMSE (partial $r_s = -0.37$, $P < 0.01$). This might be expected since AMNART and Boston naming are both verbal semantic knowledge tasks and MMSE has been shown to be correlated with education (Schmand et al., 1995). In cognitively normal subjects, AMNART acts as a measure of verbal ability and was found to be correlated with education. In cognitively impaired subjects, we found that AMNART explained variability in MMSE ($P < 0.01$), ADAS-Cog ($P = 0.02$), Boston naming ($P < 0.05$) and Trails B ($P < 0.05$). These results support the notion that AMNART explains inter-subject variation in the cognitive response to brain pathology in cognitively impaired.

## Biomarkers and cognition

In cognitively normal subjects, we did not find significant correlations between any of the biomarkers and the cognitive measures
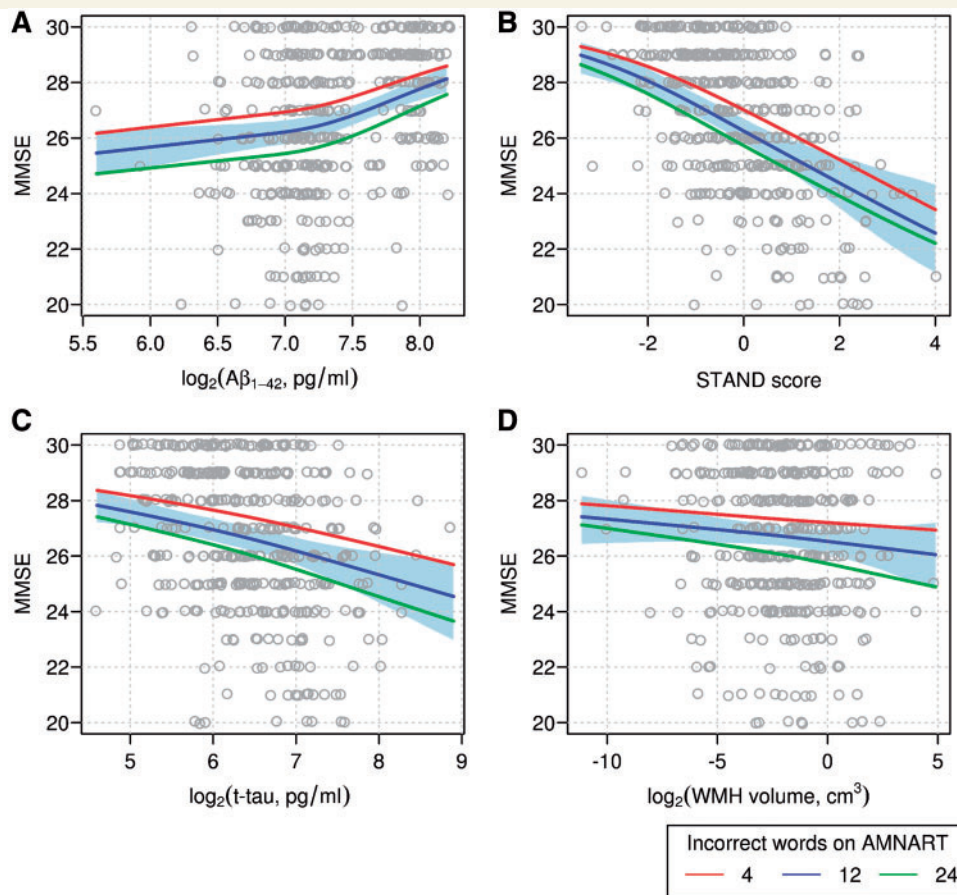
**Figure 3** Scatter plots of MMSE versus neuropathology markers: (A) CSF Amyloid-$\beta_{1-42}$ (B) STAND-score (C) t-tau and (D) WMH. Superimposed lines represent estimated mean MMSE as a function of the neuropathology marker for varying levels of AMNART. The red line represents the 15th percentile of four errors on AMNART indicating a 'good' score, the blue line represents the median of 12 errors indicating an 'average' score, and the green line represents the 85th percentile of 24 errors indicating a 'bad' score. The shaded region about the blue line indicates a 95% bootstrap confidence interval. These estimates come from penalized ordinal logistic regression models as described in the methods. STAND = Structural Abnormality Index; WMH = white matter hyperintensity.

we assessed (Auditory–Verbal Learning Test delayed recall and Boston Naming). Approximately one-third of elderly cognitively normal subjects have amyloid pathology (Katzman *et al.*, 1988; Crystal *et al.*, 1993; Hulette *et al.*, 1998; Price and Morris, 1999; Schmitt *et al.*, 2000; Morris and Price, 2001; Riley *et al.*, 2002; Knopman *et al.*, 2003). Amyloid deposition is believed to occur early in the disease process and does not directly cause clinical symptoms (Jack *et al.*, 2009; Mormino *et al.*, 2009). We, therefore, did not expect to find a strong correlation between CSF amyloid-$\beta_{1-42}$ and cognition. On the other hand, neurofibrillary tangles and neurodegeneration are believed to be downstream pathological events that progressively worsen in the presence of a relatively static total load of amyloid and which lead directly to cognitive impairment (Ingelsson *et al.*, 2004; Jack *et al.*, 2010). The absence of substantial neurodegenerative pathology in the cognitively normal subjects explains the absence of a strong correlation between biomarkers of neuronal pathology and cognition in these subjects. The literature on the lack of correlation between biomarkers of amyloid load and cognition in the cognitively normal is, however, not consistently unanimous. While some studies have found a poor correlation between amyloid load and

cognition in cognitively normal subjects (Aizenstein *et al.*, 2008; Jack *et al.*, 2009; Vemuri *et al.*, 2010) others have found significant correlations between amyloid load and cognition (Pike *et al.*, 2007; Villemagne *et al.*, 2008; Storandt *et al.*, 2009). The most logical explanation for these conflicting results is that different studies include different blends of three different groups of cognitively normal subjects: (i) normal cognition in the absence of amyloid load and neurodegeneration; (ii) normal cognition in the presence of some amyloid load and absence of neurodegeneration; and (iii) early cognitive decline in the presence of amyloid load and neurodegeneration; thus leading to different conclusions.

In cognitively impaired subjects, both biomarkers of neuronal pathology (CSF t-tau and structural MRI) and amyloid-$\beta$ amyloid load (CSF amyloid-$\beta_{1-42}$) explained variability in general cognitive performance (ADAS-Cog and MMSE). Most of the biomarkers of Alzheimer's disease correlated with the domain-specific scores as well (Auditory–Verbal Learning Test, Boston naming, Trails B) except for the lack of correlation between CSF amyloid-$\beta_{1-42}$ with Boston naming and Trails B. Our finding of stronger correlations between structural MRI and cognitive performance than between CSF measures and cognitive measures is consistent with

several recent studies (Vemuri *et al.*, 2009; Fjell *et al.*, 2010; Walhovd *et al.*, 2010). This is also consistent with a recent pathology study that found that the effect of processing resources (cognitive reserve) is slightly greater on the association between neuronal pathology and cognition than plaques and cognition (Boyle *et al.*, 2008).

In this study we found that white matter hyperintensity did not correlate with measures of general cognitive performance (ADAS-Cog and MMSE) and memory domain scores (Auditory–Verbal Learning Test) in clinically impaired subjects. However, white matter hyperintensity correlated with domain scores for language (Boston naming) and executive functioning (Trails B). Some literature has shown that the degree of white matter hyperintensity does not greatly impact cognitive performance in Alzheimer's disease (Wahlund *et al.*, 1994; Hirono *et al.*, 2000; Kono *et al.*, 2004) while others have found that the degree of white matter hyperintensity does significantly impact cognitive performance in Alzheimer's disease (DeCarli *et al.*, 1995; Fazekas *et al.*, 1996), specifically deficits in executive function and speed of cognitive processing (Brickman *et al.* 2009a; Venkatraman *et al.* 2010). These inconsistent results regarding correlations between white matter hyperintensity and general cognition measures could be because the effect of white matter hyperintensity on general cognition (measured by MMSE and ADAS-Cog) might be small and therefore differences in the population recruitment mechanisms and patient numbers may have led to different conclusions.

## Cognitive reserve and biomarkers of Alzheimer's disease pathology are independent predictors of cognitive performance

In cognitively normal subjects, since biomarkers of pathology do not explain a significant amount of variability in cognition, it is not surprising that adjusting the correlation of AMNART with Boston naming by the biomarkers does not appreciably affect the strength of this association. In cognitively impaired subjects, both biomarkers of Alzheimer's disease pathology and AMNART explained significant amount of variability in the measures of cognitive and functional performance. The strength of the partial correlation between AMNART and the cognitive measures after adjusting for each one of the biomarkers was similar to the strength of the correlation before adjustment. In the reverse analysis shown in Table 3, the strength of correlation between Alzheimer's disease biomarkers and cognitive measures was also similar before and after adjustment for AMNART. These two results taken together indicate that AMNART and biomarkers are independent predictors of cognitive performance in Alzheimer's disease.

The notion of an independent effect of AMNART on the relationship between cognition and biomarkers was further strengthened by our ordinal logistic regression findings. We found strong evidence ($P < 0.001$) that AMNART and each biomarker variable were additively associated with cognition as measured by MMSE and found no evidence for interactions between AMNART and the biomarkers ($P > 0.59$). We did not find that the effect of AMNART diminished with higher levels of pathology indicating

that an additive-protective effect of AMNART is constant across the observed range of pathological severity. Though the models summarized in Fig. 3 allow for an interaction between AMNART and biomarkers, there was very little evidence of such. This, along with the lack of rank correlation between AMNART and biomarkers, suggest that it is less an issue of being underpowered to detect the interaction than that the data are consistent with a process by which AMNART and pathology operate as largely independent but additive predictors. While the additive model is supported by our data, at some point the aggregate effects of neuropathology can be expected to dominate any neuroprotective effects afforded by cognitive reserve. Although education has been well accepted as a measure of cognitive reserve, in our preliminary analysis we found that education did not correlate with cognition after adjusting for AMNART. This suggests that AMNART may be a more robust marker of the environmental enrichment aspect of cognitive reserve than education. Rentz *et al.* (2010) also found that education does not add any (significant) information in a model of amyloid and cognition that included AMNART. This may be due to the fact that education levels do not as effectively capture the environmental enrichment afforded by life-long learning as effectively as AMNART. While other markers of cognitive reserve exist, we have only presented AMNART in this study. We also specifically tested MRI measures of total intracranial volume as an independent measure of reserve and found few associations with cognition and when present they were very weak. The 'Methods' and 'Results' are presented in the online Supplementary Material.

## Implications for the relationship between cognitive reserve, biomarkers and cognition

The key observations in this study can be summarized using Fig. 4. The cognitive decline or clinical function in mild cognitive impairment and Alzheimer's disease can be viewed as a downstream process caused by an increasing neurodegenerative pathological burden. If we plot the degree of abnormality in biomarkers and clinical function/cognition as a function of disease stage, the effect of cognitive reserve can be graphically conceptualized as moving the cognition curves (Fig. 4B, indicates a 'reference' level of cognitive reserve) relative to the biomarker curves which are located upstream. Movement of cognition relative to biomarkers due to the effect of cognitive reserve is to the left (Fig. 4A, less cognitive reserve) or right (Fig. 4C, greater cognitive reserve). If $\Delta_i$ indicates the distance between the biomarker curves and cognition for a subject with average cognitive reserve at a fixed point of cognition where $i$ denotes the different biomarkers that measure different aspects of Alzheimer's disease pathology ($i$ denotes amyloid-β load in the brain, t-tau or MRI); in this study we found evidence that the distance between both the biomarker and cognition curve is increased from $\Delta_i$ to ($\Delta_i + \lambda_{CR+}$) for subjects with high cognitive reserve and decreased from $\Delta_i$ to ($\Delta_i - \lambda_{CR-}$) in subjects with lower cognitive reserve, where $\lambda_{CR+}$ denotes the shift of the curves in subjects with high cognitive reserve and $\lambda_{CR-}$ denotes the shift in subjects with low cognitive reserve. In particular we
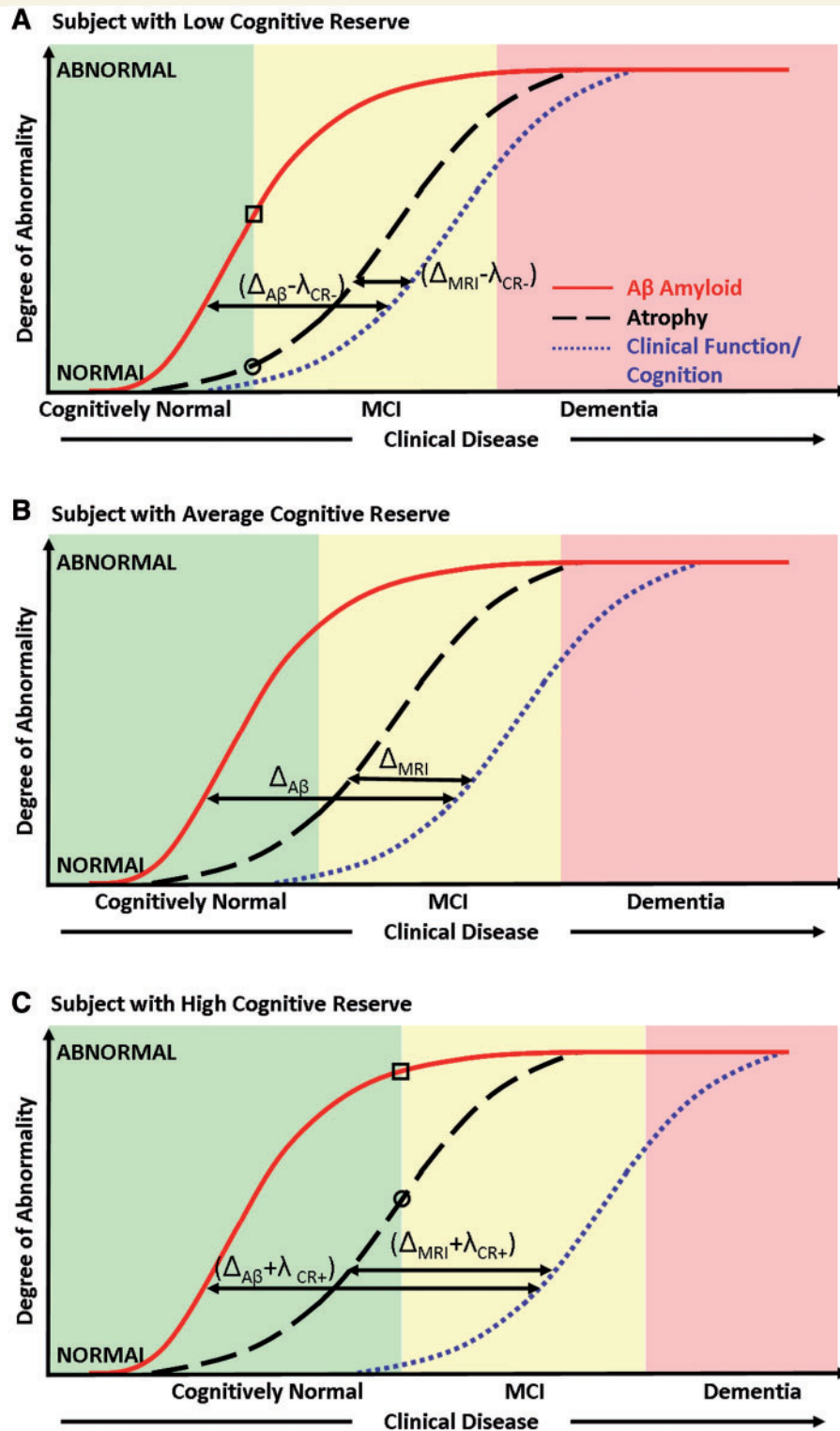
**Figure 4** Model illustrating the independent effect of cognitive reserve on the relationship between biomarkers of pathology and cognition in subjects with (**A**) low, (**B**) average and (**C**) high cognitive reserve. Clinical disease stage is indicated on the horizontal axis and the magnitude of biomarker abnormalities (from normal to maximally abnormal) on the vertical axis. The biomarker curve labels are indicated in **A**. In **A** and **C**, the levels of amyloid-β are indicated by a square and the levels of atrophy are indicated by a circle at the point where cognitively normal subjects progress to mild cognitive impairment. This illustrates that an equivalent clinical diagnostic threshold, subjects with high cognitive reserve have greater biomarker abnormalities than low cognitive reserve subjects. MCI = mild cognitive impairment.

found evidence that $\lambda_{CR+}$ and $\lambda_{CR-}$ are constants independent of the biomarker variable, i.e. the effect of cognitive reserve is additive; therefore, the distance moved by the cognition curve relative to the biomarker curves would be the same irrespective of the biomarker type. The evidence for this also comes from the fact that the strength of correlation between cognition and AMNART did not change much despite adjusting for each of the biomarkers.

A plausible model for the development of Alzheimer's disease posits that amyloid deposition occurs early in the process but by itself does not directly cause clinical symptoms (Jack *et al.*, 2009; Mormino *et al.*, 2009). On the other hand, impaired cognitive performance is largely driven by neurodegeneration that may be mediated by tau pathology. Based on this evidence, it has been hypothesized that the Alzheimer's disease pathological cascade is a roughly two-stage process where amyloidosis and neuronal pathology (tauopathy, neuronal injury and neurodegeneration) are largely sequential rather than concurrent processes (Ingelsson *et al.*, 2004; Jack *et al.*, 2009, 2010). In this analysis we also found support for this model since the correlation between MRI and cognition was stronger than the correlation between CSF amyloid-$\beta_{1-42}$ indicating that amyloid-$\beta$ levels in the brain are saturating and MRI atrophy levels evolve simultaneously with declining cognitive performance leading to a stronger correlation. This directly translates into the fact that $\Delta_{amyloid-\beta} > \Delta_{MRI}$. The primary observed effect of cognitive reserve was not to alter $\Delta_{amyloid-\beta} - \Delta_{MRI}$, but to reduce or increase the distance between the biomarker curves and cognitive performance from $\Delta_i$ to $(\Delta_i + \lambda_{CR+})$ or $(\Delta_i - \lambda_{CR-})$ based on the subject's cognitive reserve.

# Evidence for the suggested model in the literature

Two different approaches have been taken to study the effects of cognitive reserve—studies that have investigated the effect of cognitive reserve on the relationship between biomarkers and cognition or the effect of cognitive reserve on declining cognition. One class of articles provides strong evidence that biomarkers are more abnormal at a given level of cognitive performance in subjects with higher cognitive reserve when compared with subjects with lower cognitive reserve. This has been shown to be true for biomarkers of amyloid load (Kemppainen *et al.*, 2008; Roe *et al.*, 2008a; Rentz *et al.*, 2010), neurodegeneration and fibre tract integrity (Querbes *et al.*, 2009; Teipel *et al.*, 2009; Piras *et al.*, 2010), cerebral metabolism and perfusion (Alexander *et al.*, 1997; Scarmeas *et al.*, 2003; Liao *et al.*, 2005; Perneczky *et al.*, 2007; Garibotto *et al.*, 2008; Hanyu *et al.*, 2008; Cohen *et al.*, 2009) and white matter hyperintensity load (Brickman *et al.*, 2009b). Other reports support the cognitive reserve hypothesis by showing that high cognitive reserve delays the onset of Alzheimer's disease in the elderly (Hall *et al.*, 2007; Ngandu *et al.*, 2007). There was evidence for this hypothesis in our analysis where we found that better cognitive performance in both cognitively normal and cognitively impaired subjects modestly correlated with the AMNART errors after adjusting for the biomarker levels. This directly translates into the fact that at a given

level of cognitive performance the degree of biomarker abnormality is generally higher in subjects with greater cognitive reserve. This can be illustrated as follows in Fig. 4: at the conceptual point where a subject progresses from cognitively normal to mild cognitive impairment in Fig. 4A and C, the levels of biomarker abnormality (square marker for amyloid-$\beta$ levels and circle for atrophy levels) are higher in Fig. 4C (high cognitive reserve) when compared to Fig. 4A (low cognitive reserve) at the same level of cognitive performance.

A second group of articles has investigated how cognitive reserve affects the rate of cognitive decline in Alzheimer's disease. The studies that have investigated the effect of cognitive reserve on the rate of decline in Alzheimer's disease (i.e. after onset of dementia) support the hypothesis that although cognitive reserve may delay the onset of dementia, after the onset of dementia the rate of cognitive decline differs based on the subjects' cognitive reserve. Some authors have found that subjects with higher cognitive reserve decline much faster (Unverzagt *et al.*, 1998; Stern *et al.*, 1999; Andel *et al.*, 2006; Hall *et al.*, 2007; Bruandet *et al.*, 2008; Roselli *et al.*, 2009) while others have found that subjects with higher cognitive reserve decline more slowly (Fritsch *et al.*, 2001, 2002; Bennett *et al.*, 2003; Manly *et al.*, 2003; Le Carret *et al.*, 2005) when compared to subjects with low cognitive reserve. In addition, there was also evidence that the rate of decline is the same in both the groups (Del Ser *et al.*, 1999; Paradise *et al.*, 2009). We speculate that these inconsistent results may be due to the fact that subjects were sampled at different levels of biomarker abnormality along the cognition curve or at different cognitive performance at baseline resulting in differences in the observed rates. In response to increasing biomarker abnormality, there is an early increase in the slope of cognitive decline as well as an early saturation of the slope in subjects with low cognitive reserve when compared to high cognitive reserve simply due to the shift of the curves relative to the biomarker curves. This observation suggests the importance of measuring and adjusting for the degree of biomarker abnormality in order to more accurately understand the effect of cognitive reserve.

# Limitations of this study

There were several limitations of this study. First, we used AMNART which only measures one aspect of cognitive reserve, namely the pre-morbid verbal intelligence. While AMNART might not accurately capture all aspects of cognitive reserve, we believe that it provides a reasonable approximation of the beneficial effect of education and life-long learning. A related issue is that the particular aspect of cognitive reserve that AMNART is sensitive to cannot be measured perfectly. Although AMNART has been described as among the most reliable instruments in clinical use (Strauss *et al.*, 2006), and for that matter the biomarkers, are subject to various degrees of measurement error that can affect statistical inferences. Usually measurement error will attenuate associations although that is not always the case. Using the simulation-extrapolation method, our findings were largely unaffected by measurement error.

A second limitation is that ideally cognitive reserve metrics should be obtained in middle age before any disease-related

changes occur. In our data set, fewer AMNART errors were found in cognitively normal subjects than either mild cognitive impairment or Alzheimer's disease, but error rates did not differ between mild cognitive impairment and Alzheimer's disease. We, therefore, analysed cognitively normal subjects alone, and mild cognitive impairment and Alzheimer's disease as a single group. Rentz *et al*. (2010) went a step further by adjusting AMNART by MMSE to remove the confounding effect of cognitive performance on this variable. However, if we were to apply this adjustment to remove the effect of cognitive decline, we would be unable to observe a relationship between the predictor variable (AMNART adjusted for cognition) and the outcome variable (cognition) due to circularity issues. And, one of our main objectives was to evaluate the effects of AMNART and biomarkers independently on cognitive performance within clinical groups. Third, the Alzheimer's Disease Neuroimaging Initiative selection criteria excluded subjects with significant cerebrovascular disease (Hachinski score had to be <4). Therefore, the lack of a strong relationship between cognition and white matter hyperintensity in this cohort might partly be attributed to the selection criteria. Fourth, the Alzheimer's Disease Neuroimaging Initiative cohort is not population based; control and mild cognitive impairment subjects were subject to selection criteria and demented subjects were limited to mild Alzheimer's disease. The recruitment mechanisms were those used for clinical trials in Alzheimer's disease and included memory clinics, patient registries, public media campaigns and other forms of public advertisements. Consequently, inferences about the diagnostic sensitivity, specificity etc., of biomarkers in the general population cannot be drawn from Alzheimer's Disease Neuroimaging Initiative data. However, we believe that because the Alzheimer's Disease Neuroimaging Initiative incorporates patients across a cognitive spectrum ranging from normal to mild Alzheimer's disease, biologically based conclusions concerning the influence of cognitive reserve on the relationship between Alzheimer's disease biomarkers and cognition are valid. Last, the cohort we used in this study does not have autopsy confirmation, which is a limitation of almost all such observational studies.

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# Supplementary material

Supplementary material is available at *Brain* online.

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