

## Original Investigation

# Effect of Cognitive Reserve on Age-Related Changes in Cerebrospinal Fluid Biomarkers of Alzheimer Disease

Rodrigo P. Almeida, BS; Stephanie A. Schultz, BS; Benjamin P. Austin, PhD; Elizabeth A. Boots, BS; N. Maritza Dowling, PhD; Carey E. Gleason, PhD; Barbara B. Bendlin, PhD; Mark A. Sager, MD; Bruce P. Hermann, PhD; Henrik Zetterberg, MD, PhD; Cynthia M. Carlsson, MD, MS; Sterling C. Johnson, PhD; Sanjay Asthana, MD; Ozioma C. Okonkwo, PhD

**IMPORTANCE** Although advancing age is the strongest risk factor for the development of symptomatic Alzheimer disease (AD), recent studies have shown that there are individual differences in susceptibility to age-related alterations in the biomarkers of AD pathophysiology.

**OBJECTIVE** To investigate whether cognitive reserve (CR) modifies the adverse influence of age on key cerebrospinal fluid (CSF) biomarkers of AD.

**DESIGN, SETTING, AND PARTICIPANTS** A cross-sectional cohort of 268 individuals (211 in a cognitively normal group and 57 in a cognitively impaired group) from the Wisconsin Registry for Alzheimer's Prevention and the Wisconsin Alzheimer's Disease Research Center participated in this study. They underwent lumbar puncture for collection of CSF samples, from which A $\beta$ 42, total tau (t-tau), and phosphorylated tau (p-tau) were immunoassayed. In addition, we computed t-tau/A $\beta$ 42 and p-tau/A $\beta$ 42 ratios. Cognitive reserve was indexed by years of education, with 16 or more years taken to confer high reserve. Covariate-adjusted regression analyses were used to test whether the effect of age on CSF biomarkers was modified by CR. The study dates were March 5, 2010, to February 13, 2013.

**MAIN OUTCOMES AND MEASURES** Cerebrospinal fluid levels of A $\beta$ 42, t-tau, p-tau, t-tau/A $\beta$ 42, and p-tau/A $\beta$ 42.

**RESULTS** There were significant age  $\times$  CR interactions for CSF t-tau ( $\beta$  [SE] = -6.72 [2.84],  $P$  = .02), p-tau ( $\beta$  [SE] = -0.71 [0.27],  $P$  = .01), t-tau/A $\beta$ 42 ( $\beta$  [SE] = -0.02 [0.01],  $P$  = .02), and p-tau/A $\beta$ 42 ( $\beta$  [SE] = -0.002 [0.001],  $P$  = .004). With advancing age, individuals with high CR exhibited attenuated adverse alterations in these CSF biomarkers compared with individuals with low CR. This attenuation of age effects by CR tended to be more pronounced in the cognitively impaired group compared with the cognitively normal group. There was evidence of a dose-response relationship such that the effect of age on the biomarkers was progressively attenuated given additional years of schooling.

**CONCLUSIONS AND RELEVANCE** In a sample composed of a cognitively normal group and a cognitively impaired group, higher CR was associated with a diminution of age-related alterations in CSF biomarkers of AD. This suggests one pathway through which CR might favorably alter lifetime risk for symptomatic AD.

*JAMA Neurol.* 2015;72(6):699-706. doi:10.1001/jamaneurol.2015.0098  
Published online April 20, 2015.

**Author Affiliations:** Author affiliations are listed at the end of this article.

**Corresponding Author:** Ozioma C. Okonkwo, PhD, Wisconsin Alzheimer's Disease Research Center, University of Wisconsin School of Medicine and Public Health, Madison, WI 53792 (oziona@medicine.wisc.edu).

Evidence from autopsy,<sup>1</sup> epidemiological,<sup>2</sup> and cohort<sup>3</sup> studies shows that advancing age is the strongest risk factor for the accumulation of Alzheimer disease (AD)-related pathophysiological abnormalities and the ultimate development of symptomatic AD. Even so, there is significant interindividual heterogeneity in the age-specific prevalence of AD-related pathologies such that some individuals appear relatively spared the development of AD pathognomonic brain lesions even into old age.<sup>1</sup> The concept of cognitive reserve (CR) has been postulated to account not only for the mismatch between AD lesions and cognitive impairment but also for the overall decreased diathesis for these pathological changes in some individuals.<sup>1,4</sup> Essentially, the CR hypothesis posits that the intellectual enrichment that accrues from various life exposures (eg, high educational attainment and engagement in cognitively stimulating activities) lessens the adverse effect of brain pathology on cognitive function and might also attenuate the accumulation of such pathologies.<sup>4</sup>

Although AD brain abnormalities, specifically neuritic plaques and neurofibrillary tangles, have traditionally been quantified histologically at autopsy, advances in the field have made possible the in vivo measurement of biomarkers believed to reflect these underlying pathologies.<sup>5</sup> These biomarkers include cerebrospinal fluid (CSF) A $\beta$ 42, total tau (t-tau), and phosphorylated tau (p-tau), which presumptively tag cerebral A $\beta$  plaques, neuronal injury, and neurofibrillary tangles, respectively.<sup>5</sup> These biomarkers have been associated with prospective cognitive decline and risk of progressing to AD in cognitively normal individuals<sup>6,7</sup> and in those with mild cognitive impairment (MCI),<sup>8</sup> as well as with progressive cognitive deterioration and mortality in persons with probable AD dementia.<sup>9</sup>

In this study, we investigated whether educational attainment (the most widely used proxy for CR) modifies age-related alterations in these CSF biomarkers of AD. Specifically, we hypothesized that the known adverse age-dependent changes in these biomarkers will be attenuated among individuals with high educational attainment (ie, those with high CR).

## Methods

### Participants

Two hundred sixty-eight enrollees in the Wisconsin Registry for Alzheimer's Prevention and the Wisconsin Alzheimer's Disease Research Center participated in this study. The study dates were March 5, 2010, to February 13, 2013. The sample comprised 211 cognitively normal adults of late middle age enrolled in the Wisconsin Registry for Alzheimer's Prevention or the Wisconsin Alzheimer's Disease Research Center and 57 adults with cognitive impairment enrolled in the Wisconsin Alzheimer's Disease Research Center. The cognitively impaired group included 16 persons with amnesic MCI and 41 persons with mild AD. All participants were diagnostically characterized in standardized, multidisciplinary, consensus conferences: diagnoses of MCI and AD were made using applicable clinical criteria,<sup>10,11</sup> whereas cognitive normalcy was adjudicated based on intact performance on a comprehensive battery of neuropsychological tests, lack of functional im-

pairment, and absence of neurological or psychiatric conditions that might impair cognition.<sup>3</sup> Women comprised 62.3% of the sample, and the mean (SD) age of the total sample was 62.62 (8.64) years. Cognitive reserve was indexed by years of education. Individuals with fewer than 16 years of education were considered as having low CR (n = 88), whereas those with at least 16 years of education were considered as having high CR (n = 180).<sup>12,13</sup> The University of Wisconsin Institutional Review Board approved all study procedures, and each participant provided signed informed consent before participation.

### CSF Assessment

Lumbar puncture for collection of CSF samples was performed in the morning after a 12-hour fast with a Sprotte 24-gauge or 25-gauge spinal needle at L3/4 or L4/5 using gentle extraction into polypropylene syringes. Each sample consisted of 22 mL of CSF, which was then combined, gently mixed, and centrifuged at 2000g for 10 minutes. Supernatants were frozen in 0.5-mL aliquots in polypropylene tubes and stored at -80°C. The samples were immunoassayed for A $\beta$ 42, t-tau, and p-tau (at threonine 181) using enzyme-linked immunosorbent assays (INNOTEST; Fujirebio) by board-certified laboratory technicians who were blind to clinical data and used protocols accredited by the Swedish Board for Accreditation and Conformity Assessment as previously described.<sup>14</sup> Using these data, we also computed t-tau/A $\beta$ 42 and p-tau/A $\beta$ 42 ratios.

### Statistical Analysis

To investigate whether CR modifies the adverse influence of age on CSF biomarkers, we fitted a series of simultaneous entry linear regression analyses (one for each CSF biomarker) that included terms for age, sex, CR, age  $\times$  CR interaction, apolipoprotein E4 (*APOE4*) genotype (0 vs  $\geq 1$  allele), and cognitive status (ie, cognitively normal vs cognitively impaired). The age  $\times$  CR interaction term was the effect of primary interest in all models. Where significant, it would indicate a differential effect of age on CSF biomarkers as a function of CR (low vs high). Fitting our regression models via simultaneous entry ensured that the age  $\times$  CR effect (where observed) was independent of, rather than a proxy for, the influence of other covariates (eg, cognitive status or *APOE4*) on the CSF biomarkers. Correlational analyses were first used to assess the relationship between age and the CSF biomarkers. All analyses were conducted using statistical software (SPSS, version 21.0; IBM). Only findings with  $P \leq .05$  (2-tailed) were considered to be significant.

## Results

### Participant Characteristics

Table 1 lists the characteristics of the participants for the total sample and when stratified by cognitive status and CR. Within the total sample, the mean (SD) age was 62.62 (8.64) years (age range, 45-93 years), the mean (SD) years of education were 15.94 (2.44) (range, 8-21), 62.3% were women, and 45.9% were *APOE4* positive. Among the cognitively normal group, those with low CR had fewer years of education (by design), lower memory test scores, more women, and more persons with a parental

Table 1. Characteristics of Study Participants<sup>a</sup>

Characteristic	Total Sample (N = 268)	Cognitively Normal Group (n = 211)		Cognitively Impaired Group (n = 57)	
		Low CR (n = 64)	High CR (n = 147)	Low CR (n = 24)	High CR (n = 33)
Age, mean (SD) [range], y	62.62 (8.64) [45-93]	58.91 (5.98) [45-73]	59.95 (5.80) [47-72]	74.22 (8.84) [58-93]	73.29 (8.05) [58-91]
Education, mean (SD) [range], y	15.94 (2.44) [8-21]	13.25 (1.08) [12-15] <sup>b</sup>	17.33 (1.35) [16-21]	12.46 (1.67) [8-15] <sup>c</sup>	17.45 (1.50) [16-20]
MMSE score, mean (SD)	28.14 (3.11)	29.22 (1.02)	29.46 (0.72)	22.13 (4.25) <sup>c</sup>	24.70 (3.64)
Women, No. (%)	167 (62.3)	51 (79.7) <sup>b</sup>	97 (66.0)	10 (41.7)	9 (27.3)
APOE4 positive, No. (%)	123 (45.9)	26 (40.6)	62 (42.2)	15 (62.5)	20 (60.6)
Family history of dementia, No. (%)	197 (73.5)	58 (90.6) <sup>b</sup>	110 (74.8)	13 (54.2)	16 (48.5)
Clinical Dementia Rating					
Global score of 0/0.5/1.0/2.0, No.	203/47/17/1	61/3	139/8	1/13/9/1	2/23/8/0
Sum of boxes score, mean (SD)	0.78 (1.64)	0.05 (0.18)	0.06 (0.21)	4.17 (1.89) <sup>c</sup>	2.88 (1.75)
RAVLT, mean (SD)					
Total learning score	45.18 (13.96)	49.05 (8.25) <sup>b</sup>	51.69 (8.99)	21.29 (10.14) <sup>c</sup>	27.27 (6.99)
Long delay score	8.33 (4.69)	10.19 (2.75)	10.40 (3.03)	1.21 (2.38)	1.12 (1.58)
Cerebrospinal fluid biomarker, mean (SD)					
Aβ42, ng/L	672.24 (237.36)	734.32 (204.75)	733.60 (207.31)	440.21 (211.45)	442.22 (200.55)
T-tau, ng/L	389.48 (260.11)	295.26 (99.17)	304.79 (134.31)	802.62 (413.76)	648.55 (300.50)
P-tau, ng/L	47.66 (22.36)	42.14 (12.98)	41.15 (14.13)	81.50 (35.83) <sup>c</sup>	62.44 (25.38)
T-tau/Aβ42	0.74 (0.80)	0.42 (0.21)	0.44 (0.26)	2.06 (1.15)	1.74 (1.02)
P-tau/Aβ42	0.09 (0.07)	0.06 (0.03)	0.06 (0.03)	0.21 (0.11)	0.17 (0.09)

Abbreviations: CR, cognitive reserve; MMSE, Mini-Mental State Examination; RAVLT, Rey Auditory Verbal Learning Test.

<sup>a</sup> Cognitively impaired is having a diagnosis of mild cognitive impairment or probable Alzheimer dementia. Low CR is fewer than 16 years of education, and high CR is at least 16 years of education.

<sup>b</sup>  $P < .05$  for comparison between low CR and high CR groups among cognitively normal participants.

<sup>c</sup>  $P < .05$  for comparison between low CR and high CR groups among participants with cognitive impairment.

family history of dementia compared with those with high CR. Among the cognitively impaired group, those with low CR had fewer years of education (by design), higher p-tau levels, and worse Mini-Mental State Examination, Clinical Dementia Rating sum of boxes, and memory test scores compared with those with high CR. These differences were assessed at the pre-defined a level of .05 (2-tailed).

### Association Between Age and CSF Biomarkers

Pearson product moment correlations between age and CSF biomarkers within the total sample were as follows: Aβ42 ( $r = -0.30$ ,  $P < .001$ ), t-tau ( $r = 0.52$ ,  $P < .001$ ), p-tau ( $r = 0.47$ ,  $P < .001$ ), t-tau/Aβ42 ( $r = 0.48$ ,  $P < .001$ ), and p-tau/Aβ42 ( $r = 0.46$ ,  $P < .001$ ). In the cognitively normal group, the correlations were as follows: Aβ42 ( $r = 0.01$ ,  $P = .88$ ), t-tau ( $r = 0.21$ ,  $P = .002$ ), p-tau ( $r = 0.23$ ,  $P = .001$ ), t-tau/Aβ42 ( $r = 0.19$ ,  $P = .01$ ), and p-tau/Aβ42 ( $r = 0.18$ ,  $P = .01$ ). In the cognitively impaired group, the correlations were as follows: Aβ42 ( $r = 0.24$ ,  $P = .07$ ), t-tau ( $r = 0.13$ ,  $P = .35$ ), p-tau ( $r = 0.14$ ,  $P = .31$ ), t-tau/Aβ42 ( $r = -0.17$ ,  $P = .22$ ), and p-tau/Aβ42 ( $r = -0.14$ ,  $P = .29$ ). Overall, these correlations indicate that age is linked with interindividual variation in these biomarkers, as noted in prior studies.<sup>1,3,15</sup> Although unexpected, the positive correlation between age and Aβ42 within the cognitively impaired group would appear consistent with accumulating evidence that the

well-documented AD-related reduction in Aβ42 levels might be preceded by an initial phase of elevated levels.<sup>16-20</sup>

### CR and Age-Related Alterations in CSF Biomarkers

There were significant age × CR interactions for CSF t-tau, p-tau, t-tau/Aβ42, and p-tau/Aβ42 but not for Aβ42 (Table 2). To display these graphically, we followed standard procedures for generating plots for interactions between a continuous variable (ie, age) and a categorical variable (ie, CR), which entail solving the regression equation at specific anchor points on the continuous variable.<sup>21</sup> In our case, we solved the equation for age equals 50 years (younger) and age equals 80 years (older). These solutions, shown in Figure 1, revealed that adverse change in CSF biomarkers due to older age was more pronounced in individuals with low CR than in individuals with high CR. Although the age × CR interaction was not significant for Aβ42, we elected to plot the Aβ42 results for the sake of completeness. Overall, percentage reduction in the effect of older age on CSF biomarkers among the individuals with high CR vis-à-vis the individuals with low CR ranged from 72% (for p-tau) to 180% (for t-tau/Aβ42).

### Supplemental Analyses

We conducted 2 supplemental analyses to further investigate our initial age × CR findings. The first supplemental analysis

**Table 2. Modification of the Association Between Age and Cerebrospinal Fluid Biomarkers of Alzheimer Disease by Cognitive Reserve (CR)<sup>a</sup>**

Biomarker	Age × CR Interaction <sup>b</sup>		Age Effect in Low CR Group <sup>c</sup>		Age Effect in High CR Group <sup>d</sup>	
	β (SE)	P Value	β (SE)	P Value	β (SE)	P Value
Aβ42	2.25 (2.84)	.43	NA	NA	NA	NA
T-tau	-6.72 (2.84)	.02	271.96 (75.76)	<.001	70.34 (65.96)	.29
P-tau	-0.71 (0.27)	.01	29.40 (7.15)	<.001	8.19 (6.22)	.19
T-tau/Aβ42	-0.02 (0.01)	.02	0.30 (0.21)	.15	-0.24 (0.18)	.18
P-tau/Aβ42	-0.002 (0.001)	.004	0.04 (0.02)	.03	-0.02 (0.02)	.23

<sup>a</sup> Variables included in the model were age, sex, CR, an age × CR interaction, apolipoprotein E4 genotype, and cognitive status (ie, cognitively normal vs cognitively impaired). The age × CR interaction term was the effect of primary interest. The analyses were run in the total sample of cognitively normal individuals and those with cognitive impairment. Because cognitive status was adjusted for in the models, the age × CR effect is deemed to be over and above the influence of cognitive status on the cerebrospinal fluid biomarkers.

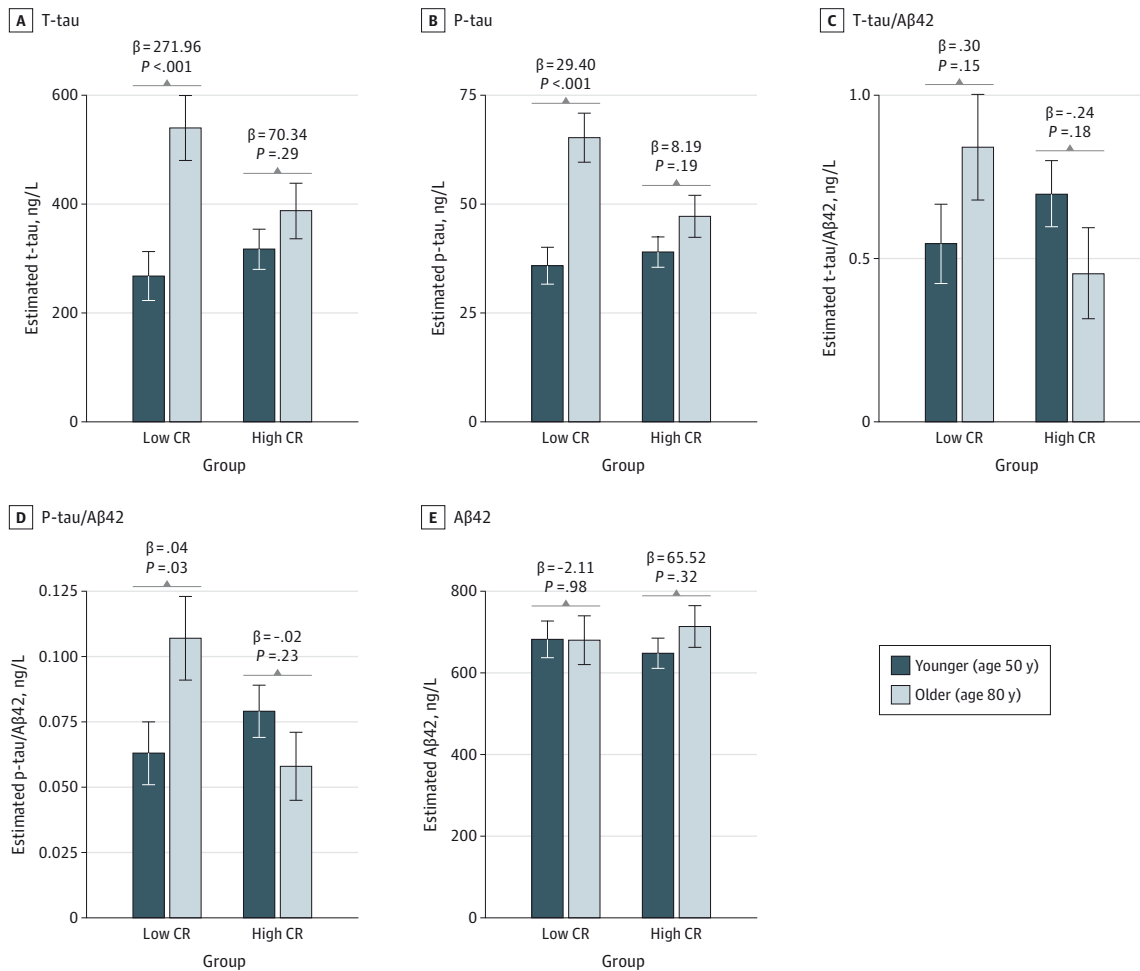
interactive term in each biomarker’s model. This term assesses whether CR modifies the effect of age on the examined biomarker.

<sup>c</sup> The regression estimates and associated P values are for the simple main effect of age on each biomarker within the low CR group.

<sup>d</sup> The regression estimates and associated P values are for the simple main effect of age on each biomarker within the high CR group.

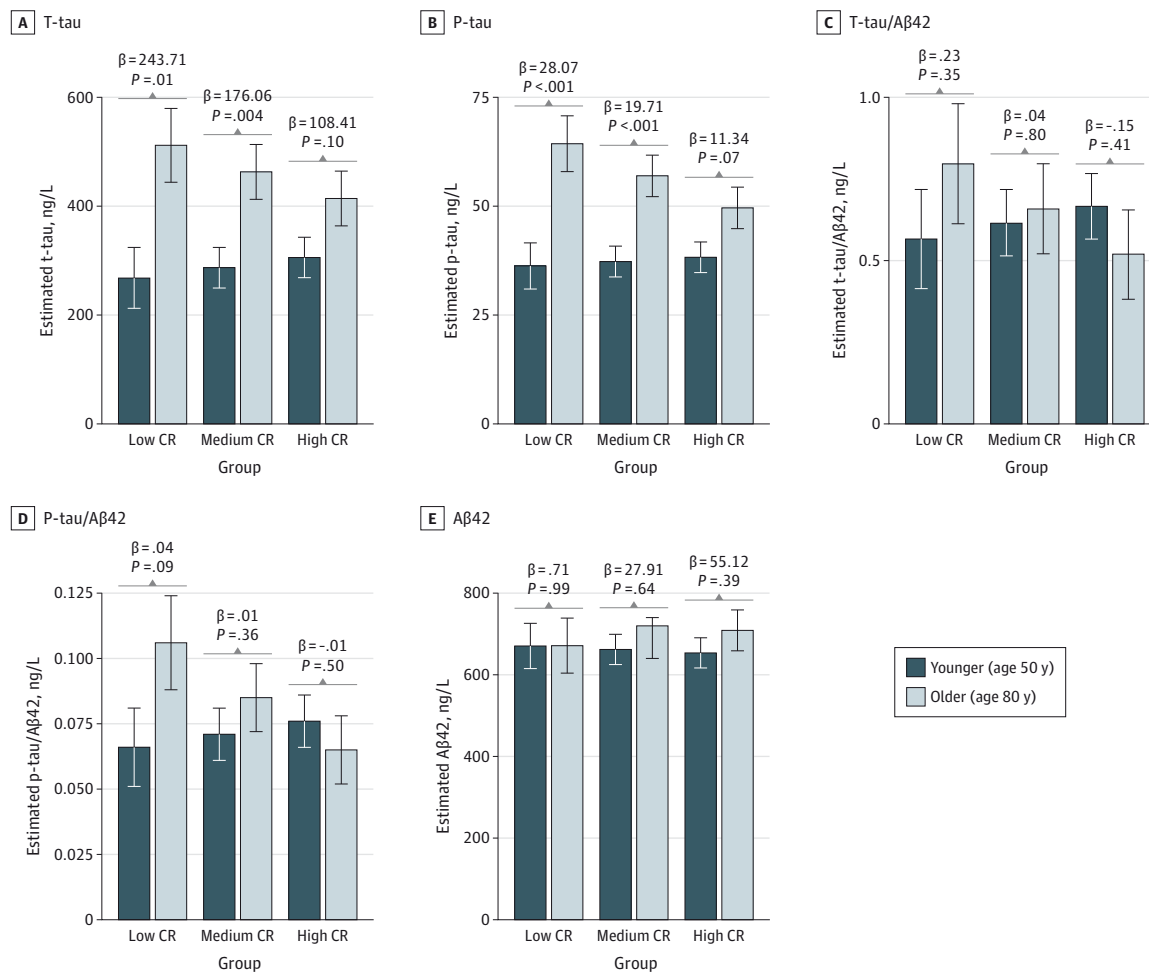
<sup>b</sup> The regression estimates and associated P values are for the age × CR

**Figure 1. Association of Higher Cognitive Reserve (CR) With Diminution of Age-Related Alterations in Cerebrospinal Fluid Biomarkers of Alzheimer Disease**



Panels A through E show adjusted means (SEs) from analyses that (within the total sample of cognitively normal individuals and those with cognitive impairment) modeled each cerebrospinal fluid biomarker as a function of age, sex, CR, an age × CR interaction, apolipoprotein E4 genotype, and cognitive status (ie, cognitively normal vs cognitively impaired). The age × CR interaction term was the effect of primary interest in all models. Because cognitive status was adjusted for in the models, the age × CR effect is deemed to be over and above the influence of cognitive status on the cerebrospinal fluid biomarkers.

Figure 2. Dose-Response in Modification of Age Effect on Cerebrospinal Fluid Biomarkers of Alzheimer Disease by Cognitive Reserve (CR)



Panels A through E show adjusted means (SEs) from analyses that (within the total sample of cognitively normal individuals and those with cognitive impairment) modeled each cerebrospinal fluid biomarker as a function of age, sex, CR, an age  $\times$  CR interaction, apolipoprotein E4 genotype, and cognitive status (ie, cognitively normal vs cognitively impaired). The age  $\times$  CR interaction term was the effect of primary interest in all models. Because cognitive status was adjusted for in the models, the age  $\times$  CR effect is deemed to be over and above the influence of cognitive status on the cerebrospinal fluid biomarkers.

was to determine whether CR modified age effects in a dose-response fashion. For this purpose, we redefined CR as low ( $\leq 12$  years of education) ( $n = 39$ ), medium (13-15 years of education) ( $n = 49$ ), or high ( $\geq 16$  years of education) ( $n = 180$ ). When our models were refit using this new CR variable, we observed near-significant age  $\times$  CR effects for p-tau ( $\beta$  [SE] =  $-0.28$  [0.16],  $P = .08$ ) and p-tau/A $\beta$ 42 ( $\beta$  [SE] =  $-0.001$  [0.001],  $P = .05$ ), trending effects for t-tau ( $\beta$  [SE] =  $-2.26$  [1.70],  $P = .19$ ) and t-tau/A $\beta$ 42 ( $\beta$  [SE] =  $-0.01$  [0.01],  $P = .17$ ), and a nonsignificant effect for A $\beta$ 42 ( $\beta$  [SE] =  $0.91$  [1.68],  $P = .59$ ). Simple main effect analyses revealed evidence for a dose-response relationship, wherein the adverse effect of age on the biomarkers was progressively diminished at higher CR levels (Figure 2). When education was treated as a continuous variable, a significant age  $\times$  CR interaction was only seen for p-tau/A $\beta$ 42 ( $P = .03$ ), with trends for p-tau ( $P = .12$ ) and t-tau/A $\beta$ 42 ( $P = .17$ ). When low vs high CR was defined as 12 or fewer vs more than 12 years of education, there were no significant age  $\times$  CR interactions ( $P \geq .47$  for all biomarkers).

The second supplemental analysis was performed to ascertain whether modification of age-related changes in CSF biomarkers by CR was similarly instantiated in the cognitively normal and cognitively impaired groups. To do this, we refit the original regression models with the inclusion of an age  $\times$  CR  $\times$  cognitive status interaction term. A statistically significant age  $\times$  CR  $\times$  cognitive status term would indicate that the age  $\times$  CR interaction was differentially instantiated in each group, whereas a nonsignificant term would indicate equivalence of instantiation. For this 3-way interaction term to be properly parameterized, it was necessary to also include CR  $\times$  cognitive status and age  $\times$  cognitive status interaction terms in the regression models so as to completely elucidate all 2-way interactions (ie, age  $\times$  CR, CR  $\times$  cognitive status, and age  $\times$  cognitive status) that are nested within the 3-way interaction (ie, age  $\times$  CR  $\times$  cognitive status).

We observed significant age  $\times$  CR  $\times$  cognitive status interactions for p-tau ( $\beta$  [SE] =  $-1.63$  [0.75],  $P = .03$ ), t-tau/A $\beta$ 42 ( $\beta$  [SE] =  $-0.05$  [0.02],  $P = .01$ ), and p-tau/A $\beta$ 42 ( $\beta$  [SE] =  $-0.010$  [0.002],

$P = .01$ ). The negative sign of the regression estimates in these findings indicates that the attenuation of age-related changes in CSF biomarkers among those with high CR was (comparatively speaking) more pronounced in the cognitively impaired group relative to the cognitively normal group. That is, high CR exerted a relatively greater abatement of age-related changes in CSF biomarkers in the cognitively impaired group compared with the cognitively normal group. However, when the 3-way age  $\times$  CR  $\times$  cognitive status interactions were formally decomposed into within-group age  $\times$  CR interactions, they failed to attain statistical significance in either group, with the possible exception of p-tau/A $\beta$ 42, which neared significance in the cognitively impaired group ( $\beta$  [SE] =  $-0.55$  [0.31],  $P = .08$ ) but not in the cognitively normal group ( $\beta$  [SE] =  $-0.38$  [0.26],  $P = .14$ ). Nonetheless, all within-group age  $\times$  CR interactions were in the same direction, which provided reassurance of their consistency. The failure of the within-group age  $\times$  CR interactions to reach significance was due to reduced power or comparatively restricted range in the distribution of age, education, and the CSF biomarkers within each group.

## Discussion

In this study, we investigated whether CR modifies age-related alterations in CSF biomarkers of AD in a cohort comprising a cognitively normal group and a cognitively impaired group. With advancing age, individuals with high CR (ie,  $\geq 16$  years of education) exhibited less adverse alterations in CSF t-tau, p-tau, t-tau/A $\beta$ 42, and p-tau/A $\beta$ 42 compared with individuals with low CR. Follow-up analyses revealed that this modification of age-biomarker associations by CR was comparatively more pronounced among the cognitively impaired group relative to the cognitively normal group. To our knowledge, this study is the first to adopt this novel approach (ie, the attenuation of the effect of age on AD biomarkers) to the examination of CR. The present observations are consistent with a recent study<sup>3</sup> from our group showing that increased physical activity ameliorates the influence of age on key AD imaging biomarkers such as amyloid burden, glucose metabolism, and hippocampal volume.

In a landmark study, Braak and Braak<sup>1</sup> investigated the age-dependent evolution of extracellular amyloid deposits and intraneuronal neurofibrillary changes via assessment of a large set of nonselected autopsy cases using a staging heuristic. They observed that there was appreciable interindividual heterogeneity in the prevalence of these hallmark AD brain lesions such that some individuals showed much less pathology than would be expected for their age, suggesting that neither amyloid deposits nor neurofibrillary tangles necessarily accompany old age.<sup>1</sup> By showing that high CR is related to reduced age-related changes in CSF biomarkers of AD and (by proxy) to less AD-related brain pathology, our present findings suggest an important role for CR as a modifier of the evolution of AD pathophysiological changes.

Studies of CR have traditionally framed the construct in terms of the potential for certain life experience to alter the association between brain pathology and clinical outcome. For example, in a sample composed of cognitively normal persons and those with

dementia, Roe et al<sup>12</sup> investigated whether, given elevated cerebral fibrillar amyloid, individuals who had attained more years of schooling (their proxy for CR) exhibited preserved cognitive function compared with individuals who had fewer years of schooling. Across all cognitive outcomes examined, they found that the negative effect of increased fibrillar amyloid on cognition was diminished among those with higher educational attainment. In a similarly designed study, Rentz et al<sup>22</sup> examined whether CR, as measured by the American National Adult Reading Test,<sup>23</sup> modifies associations between amyloid deposition and impaired cognition in a pooled sample of persons with dementia and nondemented individuals. They found evidence of such effect modification. Specifically, greater amyloid deposition was only associated with worse cognition at lower CR levels. At higher CR levels, this association was nonexistent. In a large, multiethnic study, Yaffe and colleagues<sup>24</sup> similarly found that the relationship between plasma A $\beta$ 42/40 and 9-year decline in Modified Mini-Mental State Examination scores was pronounced in those with low educational attainment but was attenuated in those with higher educational attainment. There is also evidence that higher educational attainment likewise diminishes the effect of cerebral atrophy and ischemic damage on cognitive decline.<sup>25,26</sup>

Other studies<sup>13,27-31</sup> have investigated the beneficial effect of CR from a different perspective. Rather than model the potential for CR to modify the association between pathology and cognition, the studies instead present evidence that (for a given level of cognitive function) individuals with higher CR harbor greater brain pathology and are therefore further along the disease continuum than those with lower CR. For example, Kempainen et al<sup>13</sup> found that, when matched for degree of cognitive impairment and other relevant covariates, highly educated patients with mild AD harbored greater fibrillar amyloid and lower glucose metabolic rate compared with those who had fewer years of schooling. In a prospective study, Rolstad and colleagues<sup>31</sup> found that patients with MCI with higher education had lower A $\beta$ 42 at baseline and experienced greater drop in A $\beta$ 42 longitudinally compared with those with less education. In contrast with these foregoing approaches to investigating CR, we present novel evidence that greater CR might attenuate the prototypical age-dependent dynamics of AD biomarkers in CSF. Differences in approach notwithstanding, the reports from these studies and ours provide convergent support for the CR hypothesis and underscore the putative role that certain life exposures might have in forestalling or retarding the AD cascade.

Even so, our observation that CR modifies age-related changes in CSF biomarkers was made for t-tau and p-tau but not for A $\beta$ 42, the canonical biomarker of AD pathophysiology.<sup>32</sup> It is not entirely clear why this is so. It perhaps suggests that CR exerts a stronger influence on neurodegeneration compared with amyloidosis. Consistent with this hypothesis, other studies<sup>33,34</sup> have found that, whereas CR interacted with t-tau and p-tau to predict incident cognitive impairment in preclinical AD, such an interaction was not observed for A $\beta$ 42. One potential caveat to this interpretation is that, although CR did not modify the association between age and A $\beta$ 42 in our study, it modified the association between age and t-tau/A $\beta$ 42 and p-tau/A $\beta$ 42. Furthermore, compared with isolated amyloidosis, coexistent abnormality in t-tau or p-tau and A $\beta$ 42 is generally con-

sidered to be more prototypical of an AD syndrome across the disease spectrum.<sup>5-9</sup>

We observed a significant age × CR × cognitive status interaction, indicating that modification of the relationship between age and the CSF biomarkers by CR was more pronounced in the cognitively impaired group compared with the cognitively normal group. This is a notable finding and, although its full meaning might be open to question, appears consistent with reports in related areas of inquiry suggesting that the protective effect of various life exposures vis-à-vis AD risk is more marked among individuals with greater vulnerability. For example, recent data from a large population-based study<sup>35</sup> revealed that the decreased risk of dementia as a function of higher educational attainment was more striking among those who were *APOE4* positive. Similarly, engagement in physical activity during midlife has been shown to be differentially associated with a reduced risk of dementia among *APOE4* carriers.<sup>36</sup>

An important limitation of our study is its cross-sectional nature. Although we have estimated how CR might modify age-related alterations in CSF biomarkers using statistical approaches, prospective designs would be better suited for an in-depth examination of this scientific question. We also acknowledge that, although education is the most commonly used proxy for CR, education is arguably a multifactorial variable with wide ranging interrelationships with various factors. For example, lower education is associated with

an array of cardiovascular morbidity, including obesity,<sup>37</sup> heart disease,<sup>38</sup> and stroke,<sup>38,39</sup> each of which is associated with increased risk of dementia in general and AD in particular.<sup>40</sup> Therefore, it is possible that education may reduce risk of AD through mechanisms that are not directly related to CR. Relatedly, education performs closely with other indexes of socioeconomic status (eg, income and occupational attainment) that have been shown to be related to risk of AD.<sup>4</sup> However, existent evidence suggests that the association between education and risk of AD persists even after adjusting for socioeconomic indicators, medical comorbidities, intelligence, and various lifestyle factors.<sup>41,42</sup>

## Conclusions

In summary, this study demonstrates that high CR blunts the characteristic age-related dynamic changes in CSF biomarkers of AD, suggesting a pathway through which CR might favorably alter lifetime risk for symptomatic AD. This finding takes on considerable import when placed against the backdrop of ongoing global efforts to thwart a looming AD epidemic that is driven by the rapid expansion in the elderly segment of the world's population.<sup>43</sup> Well-designed longitudinal studies will be the logical next step for more rigorously testing these exciting hypotheses.

### ARTICLE INFORMATION

**Accepted for Publication:** January 23, 2015.

**Published Online:** April 20, 2015.

doi:10.1001/jamaneurol.2015.0098.

**Author Affiliations:** Currently a student at Fluminense Federal University, Niteroi, Brazil (Almeida); Geriatric Research Education and Clinical Center, William S. Middleton Memorial Veterans Hospital, Madison, Wisconsin (Almeida, Schultz, Austin, Boots, Gleason, Bendlin, Carlsson, Johnson, Asthana, Okonkwo); Wisconsin Alzheimer's Institute, University of Wisconsin School of Medicine and Public Health, Madison (Almeida, Schultz, Boots, Bendlin, Sager, Hermann, Johnson, Asthana, Okonkwo); Wisconsin Alzheimer's Disease Research Center, University of Wisconsin School of Medicine and Public Health, Madison (Almeida, Schultz, Austin, Boots, Dowling, Gleason, Bendlin, Sager, Hermann, Carlsson, Johnson, Asthana, Okonkwo); Department of Biostatistics and Medical Informatics, University of Wisconsin School of Medicine and Public Health, Madison (Dowling); Department of Neurology, University of Wisconsin School of Medicine and Public Health, Madison (Dowling, Hermann); Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at University of Gothenburg, Mölndal, Sweden (Zetterberg); University of London Institute of Neurology, Queen Square, London, England (Zetterberg).

**Author Contributions:** Dr Okonkwo had full access to all the data in this study and takes responsibility for the integrity of the data and the accuracy of the data analyses.

**Study concept and design:** Almeida, Schultz, Okonkwo.

**Acquisition, analysis, or interpretation of data:** All authors.

**Drafting of the manuscript:** Almeida, Schultz, Okonkwo.

**Critical revision of manuscript for important intellectual content:** All authors.

**Statistical analysis:** Almeida, Schultz, Okonkwo.

**Obtained funding:** Sager, Carlsson, Johnson, Asthana, Okonkwo.

**Administrative, technical, or material support:** Austin, Boots, Dowling, Gleason, Bendlin, Sager, Hermann, Zetterberg.

**Study supervision:** Okonkwo.

**Conflict of Interest Disclosures:** None reported.

**Funding/Support:** This work was supported by grants K23 AG045957 (Dr Okonkwo), R01 AG031790 (Dr Carlsson), R01 AG021155 and R01 AG027161 (Dr Johnson), and P50 AG033514 (Dr Asthana) from the National Institute on Aging and by Clinical and Translational Science Award UL1RRO25011 to the University of Wisconsin, Madison. Portions of this research were supported by the Wisconsin Alumni Research Foundation, Helen Bader Foundation, Northwestern Mutual Foundation, and Extencicare Foundation, as well as by the US Department of Veterans Affairs, including facilities and resources at the Geriatric Research Education and Clinical Center of the William S. Middleton Memorial Veterans Hospital, Madison. Mr Almeida's yearlong study abroad at the University of Wisconsin, Madison, was funded by a scholarship from the Coordination for the Improvement of Higher Education Personnel (CAPES) Foundation.

**Role of the Funder/Sponsor:** The funders had no role with respect to design and conduct of the study; collection, management, analysis, and

interpretation of the data; preparation, review, or approval of the manuscript; and the decision to submit the manuscript for publication.

**Additional Contributions:** We acknowledge the researchers and staff of the Clinical Neurochemistry Laboratory, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at University of Gothenburg, Mölndal, Sweden, where the cerebrospinal fluid assays were performed. Importantly, we thank study participants in the Wisconsin Registry for Alzheimer's Prevention and the Wisconsin Alzheimer's Disease Research Center, without whom this work would not be possible.

### REFERENCES

- Braak H, Braak E. Frequency of stages of Alzheimer-related lesions in different age categories. *Neurobiol Aging*. 1997;18(4):351-357.
- Hebert LE, Scherr PA, Beckett LA, et al. Age-specific incidence of Alzheimer's disease in a community population. *JAMA*. 1995;273(17):1354-1359.
- Okonkwo OC, Schultz SA, Oh JM, et al. Physical activity attenuates age-related biomarker alterations in preclinical AD. *Neurology*. 2014;83(19):1753-1760.
- Stern Y. Cognitive reserve in ageing and Alzheimer's disease. *Lancet Neurol*. 2012;11(11):1006-1012.
- Shaw LM, Vanderstichele H, Knopik-Czajka M, et al. Alzheimer's Disease Neuroimaging Initiative. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. *Ann Neurol*. 2009;65(4):403-413.
- Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM. Cerebrospinal fluid

- tau/ $\beta$ -amyloid<sub>42</sub> ratio as a prediction of cognitive decline in nondemented older adults. *Arch Neurol*. 2007;64(3):343-349.
7. Vos SJ, Xiong C, Visser PJ, et al. Preclinical Alzheimer's disease and its outcome: a longitudinal cohort study. *Lancet Neurol*. 2013;12(10):957-965.
  8. Okonkwo OC, Mielke MM, Griffith HR, et al; Alzheimer's Disease Neuroimaging Initiative. Cerebrospinal fluid profiles and prospective course and outcome in patients with amnesic mild cognitive impairment. *Arch Neurol*. 2011;68(1):113-119.
  9. Wallin AK, Blennow K, Zetterberg H, Londos E, Minthon L, Hansson O. CSF biomarkers predict a more malignant outcome in Alzheimer disease. *Neurology*. 2010;74(19):1531-1537.
  10. Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. 2011;7(3):270-279.
  11. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. 2011;7(3):263-269.
  12. Roe CM, Mintun MA, D'Angelo G, Xiong C, Grant EA, Morris JC. Alzheimer disease and cognitive reserve: variation of education effect with carbon 11-labeled Pittsburgh Compound B uptake. *Arch Neurol*. 2008;65(11):1467-1471.
  13. Kempainen NM, Aalto S, Karrasch M, et al. Cognitive reserve hypothesis: Pittsburgh Compound B and fluorodeoxyglucose positron emission tomography in relation to education in mild Alzheimer's disease. *Ann Neurol*. 2008;63(1):112-118.
  14. Palmqvist S, Zetterberg H, Blennow K, et al. Accuracy of brain amyloid detection in clinical practice using cerebrospinal fluid  $\beta$ -amyloid 42: a cross-validation study against amyloid positron emission tomography. *JAMA Neurol*. 2014;71(10):1282-1289.
  15. Jack CR Jr, Wiste HJ, Weigand SD, et al. Age-specific population frequencies of cerebral  $\beta$ -amyloidosis and neurodegeneration among people with normal cognitive function aged 50-89 years: a cross-sectional study. *Lancet Neurol*. 2014;13(10):997-1005.
  16. Reiman EM, Quiroz YT, Fleisher AS, et al. Brain imaging and fluid biomarker analysis in young adults at genetic risk for autosomal dominant Alzheimer's disease in the presenilin 1 E280A kindred: a case-control study. *Lancet Neurol*. 2012;11(12):1048-1056.
  17. Schupf N, Tang MX, Fukuyama H, et al. Peripheral A $\beta$  subspecies as risk biomarkers of Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2008;105(37):14052-14057.
  18. Mayeux R, Honig LS, Tang MX, et al. Plasma A $\beta$ 40 and A $\beta$ 42 and Alzheimer's disease: relation to age, mortality, and risk. *Neurology*. 2003;61(9):1185-1190.
  19. Assini A, Cammarata S, Vitali A, et al. Plasma levels of amyloid beta-protein 42 are increased in women with mild cognitive impairment. *Neurology*. 2004;63(5):828-831.
  20. Näslund J, Haroutunian V, Mohs R, et al. Correlation between elevated levels of amyloid  $\beta$ -peptide in the brain and cognitive decline. *JAMA*. 2000;283(12):1571-1577.
  21. Tabachnick BG, Fidell LS. *Using Multivariate Statistics*. 5th ed. New York, NY: Harper Collins; 2007.
  22. Rentz DM, Locascio JJ, Becker JA, et al. Cognition, reserve, and amyloid deposition in normal aging. *Ann Neurol*. 2010;67(3):353-364.
  23. Ryan JJ, Paolo AM. A screening procedure for estimating premorbid intelligence in the elderly. *Clin Neuropsychol*. 1992;6:53-62.
  24. Yaffe K, Weston A, Graff-Radford NR, et al. Association of plasma  $\beta$ -amyloid level and cognitive reserve with subsequent cognitive decline. *JAMA*. 2011;305(3):261-266.
  25. Mortamais M, Portet F, Brickman AM, et al. Education modulates the impact of white matter lesions on the risk of mild cognitive impairment and dementia. *Am J Geriatr Psychiatry*. 2014;22(11):1336-1345.
  26. Pernecky R, Wagenpfeil S, Lunetta KL, et al. Education attenuates the effect of medial temporal lobe atrophy on cognitive function in Alzheimer's disease: the MIRAGE study. *J Alzheimers Dis*. 2009;17(4):855-862.
  27. Garibotto V, Borroni B, Kalbe E, et al. Education and occupation as proxies for reserve in aMCI converters and AD: FDG-PET evidence. *Neurology*. 2008;71(17):1342-1349.
  28. Pernecky R, Drzezga A, Diehl-Schmid J, et al. Schooling mediates brain reserve in Alzheimer's disease: findings of fluoro-deoxy-glucose-positron emission tomography. *J Neurol Neurosurg Psychiatry*. 2006;77(9):1060-1063.
  29. Ewers M, Insel PS, Stern Y, Weiner MW; Alzheimer's Disease Neuroimaging Initiative (ADNI). Cognitive reserve associated with FDG-PET in preclinical Alzheimer disease. *Neurology*. 2013;80(13):1194-1201.
  30. Stern Y, Alexander GE, Prohovnik I, Mayeux R. Inverse relationship between education and parietotemporal perfusion deficit in Alzheimer's disease. *Ann Neurol*. 1992;32(3):371-375.
  31. Rolstad S, Nordlund A, Eckerström C, Gustavsson MH, Zetterberg H, Wallin A. Cognitive reserve in relation to A $\beta$ 42 in patients converting from MCI to dementia: a follow-up report. *Dement Geriatr Cogn Disord*. 2009;28(2):110-115.
  32. Jack CR Jr, Knopman DS, Jagust WJ, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol*. 2013;12(2):207-216.
  33. Soldan A, Pettigrew C, Li S, et al; BIOCARD Research Team. Relationship of cognitive reserve and cerebrospinal fluid biomarkers to the emergence of clinical symptoms in preclinical Alzheimer's disease. *Neurobiol Aging*. 2013;34(12):2827-2834.
  34. Roe CM, Fagan AM, Grant EA, et al. Cerebrospinal fluid biomarkers, education, brain volume, and future cognition. *Arch Neurol*. 2011;68(9):1145-1151.
  35. Wang HX, Gustafson DR, Kivipelto M, et al. Education halves the risk of dementia due to apolipoprotein  $\epsilon$ 4 allele: a collaborative study from the Swedish Brain Power initiative. *Neurobiol Aging*. 2012;33(5):1007.e1-1007.e7.
  36. Rovio S, Kåreholt I, Helkala EL, et al. Leisure-time physical activity at midlife and the risk of dementia and Alzheimer's disease. *Lancet Neurol*. 2005;4(11):705-711.
  37. Kaplan GA, Keil JE. Socioeconomic factors and cardiovascular disease: a review of the literature. *Circulation*. 1993;88(4, pt 1):1973-1998.
  38. Liu K, Cedres LB, Stamler J, et al. Relationship of education to major risk factors and death from coronary heart disease, cardiovascular diseases and all causes: findings of three Chicago epidemiologic studies. *Circulation*. 1982;66(6):1308-1314.
  39. Wong MD, Shapiro MF, Boscardin WJ, Ettner SL. Contribution of major diseases to disparities in mortality. *N Engl J Med*. 2002;347(20):1585-1592.
  40. Gorelick PB, Scuteri A, Black SE, et al; American Heart Association Stroke Council, Council on Epidemiology and Prevention, Council on Cardiovascular Nursing, Council on Cardiovascular Radiology and Intervention, and Council on Cardiovascular Surgery and Anesthesia. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2011;42(9):2672-2713.
  41. McDowell I, Xi G, Lindsay J, Tierney M. Mapping the connections between education and dementia. *J Clin Exp Neuropsychol*. 2007;29(2):127-141.
  42. Ngandu T, von Strauss E, Helkala EL, et al. Education and dementia: what lies behind the association? *Neurology*. 2007;69(14):1442-1450.
  43. Khachaturian ZS, Petersen RC, Snyder PJ, et al. Developing a global strategy to prevent Alzheimer's disease: Leon Thal Symposium 2010. *Alzheimers Dement*. 2011;7(2):127-132.