Neuropsychology

Alzheimer Disease Biomarkers, Attentional Control, and Semantic Memory Retrieval: Synergistic and Mediational Effects of Biomarkers on a Sensitive Cognitive Measure in Non-Demented Older Adults

Andrew J. Aschenbrenner, David A. Balota, Chi-Shing Tse, Anne M. Fagan, David M. Holtzman, Tammie L. S. Benzinger, and John C. Morris

Online First Publication, September 15, 2014. http://dx.doi.org/10.1037/neu0000133

Neuropsychology

0894-4105/14/$12.00 http://dx.doi.org/10.1037/neu0000133

Alzheimer Disease Biomarkers, Attentional Control, and Semantic Memory Retrieval: Synergistic and Mediational Effects of Biomarkers on a Sensitive Cognitive Measure in Non-Demented Older Adults

Andrew J. Aschenbrenner and David A. Balota
Washington University in St. Louis

Chi-Shing Tse
The Chinese University of Hong Kong

Anne M. Fagan, David M. Holtzman, Tammie L. S. Benzinger, and John C. Morris
Washington University in St. Louis

Objective: Past studies have shown that measures of attentional control and semantic memory are sensitive markers of Alzheimer's disease (AD). The effects of established biomarkers of AD (cerebrospinal fluid tau and amyloid-beta42, positron emission tomography Pittsburgh compound-B, and apolipoprotein E [APOE] genotype) on concurrent cognitive performance in cognitively normal individuals have been mixed. The present study examined the utility of combining attentional control with semantic retrieval as a sensitive correlate of AD biomarkers and used mediation analyses to examine possible mechanisms by which the biomarkers influence cognition. ***Method:*** Three hundred sixty-three participants completed a category verification task (CVT), and 113 of them concurrently underwent biomarker assessments. On each trial, participants viewed a category (e.g., "unit of time") and verified whether a subsequent target item was an exemplar of the category ("hour") or not ("clock"). Importantly, the nonmembers of the category were associatively related to the category (e.g., "clock" is not "a unit of time," but is highly related), and demanded attentional control to reject. ***Results:*** Accuracy to the foil items was the strongest discriminator between healthy aging and very mild symptomatic AD. Cerebrospinal fluid biomarkers had independent yet synergistic influence on CVT performance in cognitively healthy older adults. Furthermore, the influence of the biomarkers and APOE genotype was mediated primarily through increased levels of PIB. ***Conclusion:*** The combined influence of attentional control with semantic retrieval is a marker of symptomatic AD and a sensitive correlate of established biomarkers for AD risk in cognitively healthy participants. The biomarkers influenced cognition primarily through increased levels of amyloid in the brain.

Keywords: Alzheimer's disease, biomarkers, attention, semantic retrieval

There has been considerable research focusing on aspects of cognitive performance that can best discriminate healthy aging from the earliest, detectable, symptomatic stages of Alzheimer's disease (AD). It is now well established that AD-related changes are present in the brain long before the manifestation of overt cognitive symptoms (Morris et al., 1996; Price et al., 2009), which suggests a lengthy prodromal stage of the disease. Thus, it is critical to explore markers that are indicative of this presymptomatic stage.

Several biological markers have already been identified. For example, the presence of at least one apolipoprotein ε4 allele is a well-known genetic risk factor (Blacker et al., 1997). More recently, changes in levels of amyloid-beta 42 (Aβ42), tau, and phospho-tau181 (p-tau$_{181}$) in the cerebrospinal fluid (CSF) have been shown to be sensitive presymptomatic markers (Diniz, Pinto Jr., & Forlenza, 2008; Fagan et al., 2007). Fagan et al. (2007) reported levels of CSF tau and p-tau$_{181}$ increase (markers of neurodegeneration and/or neurofibrillary tangles) while levels of Aβ42 decrease (a marker of amyloid deposition) in the earliest stages of AD. In addition, it is also possible to directly image amyloid plaques in the brain using positron emission tomography (PET) and the amyloid tracer, Pittsburgh compound-B (PIB). Individuals with symptomatic AD exhibit greater levels of PIB retention compared to healthy controls, particularly in prefrontal and temporal brain regions (Klunk et al., 2004; Mintun et al.,

Andrew J. Aschenbrenner and David A. Balota, Department of Psychology, Washington University in St. Louis; Chi-Shing Tse, Department of Educational Psychology, The Chinese University of Hong Kong; Anne M. Fagan and David M. Holtzman, Department of Neurology and The Knight Alzheimer's Disease Research Center, Washington University in St. Louis; Tammie L. S. Benzinger, The Knight Alzheimer's Disease Research Center and Department of Radiology, Washington University in St. Louis; John C. Morris, Department of Neurology and The Knight Alzheimer's Disease Research Center, Washington University in St. Louis.

We thank the participants, the Clinical Core of the Knight Alzheimer's Disease Research Center at Washington University for providing their clinical evaluations, and the Genetics Core for providing APOE genotyping. Funding support was provided by the National Institute of Aging (NIA; P50 AG05681, P01AG03991, P01AG026276 and T32 AG000030-32).

Correspondence concerning this article should be addressed to David A. Balota, Department of Psychology, Washington University, St. Louis, MO 63130. E-mail: dbalota@wustl.edu

2006). Importantly, both CSF measures and PIB have also been shown to accumulate in some cognitively healthy older adults (Fagan et al., 2009; Morris et al., 2010).

Given the potential importance of these biomarkers for the early detection of AD, many studies have examined the correlations among the biomarkers to gain a finer understanding of early pathology (e.g., Jagust et al., 2009). Recent modeling efforts have supported the hypothesis that multiple, independent processes contribute to the pathogenesis of AD (e.g., Mungas, Tractenberg, Schneider, Crane & Bennett, 2014; Storandt, Head, Fagan, Holtzman & Morris, 2012). For example, using hierarchical modeling, Storandt et al. (2012) demonstrated both tau and Aβ42 account for independent variance in fibrillar amyloid burden measured with PET PIB. Specifically, PIB is negatively correlated with Aβ42 and positively correlated with tau. Furthermore, the two CSF measures also interact such that Aβ42 is more strongly correlated with PIB when tau levels are high. These results suggest that an early event in AD pathogenesis is the sequestering of amyloid into plaques in the brain that reduces the levels of Aβ42 in the CSF and increases PIB binding (Fagan et al., 2009). This may subsequently lead to cell death, which may reflect the elevated levels of tau. However, the fact that the CSF markers are uncorrelated with each other, and are also uncorrelated with brain volume, led Storandt et al. to suggest there may be at least three independent processes that contribute to AD pathology prior to the onset of clinically detectable symptoms.

However, the question remains as to whether multiple mechanisms also contribute separately to presymptomatic changes in *cognitive* outcomes, or if instead only the joint effects of multiple biomarkers have any detectable influence on cognition. Specifically, one might expect the CSF biomarkers to operate entirely through their joint relationship with PIB. In this scenario, PIB should completely mediate any observed relationship between CSF biomarkers and cognitive performance. However, it is also possible that CSF biomarkers may have independent effects above and beyond those due to PIB, which would suggest changes in CSF markers reflect additional processes unrelated to amyloid deposition measured with PIB. Such questions have been difficult to address, in part because relationships between the biomarkers and concurrent cognitive performance have been difficult to establish.

Importantly, in the results of Storandt et al. (2012), none of the analyzed biomarkers were related to outcomes on standard psychometric tests. Indeed, although these biomarkers have been shown to predict future cognitive decline in both cognitively healthy controls and individuals with mild cognitive impairment (Doraiswamy et al., 2012; Fagan et al., 2007; Roe et al., 2013; Storandt, Mintun, Head, & Morris, 2009), relatively few studies have reported a relationship between biomarkers and concurrent cognitive function. For example, Duchek et al. (2009) investigated the effects of several AD biomarkers on performance in standard attentional control tasks (Stroop, Simon, and task switching), and their results indicated intraindividual variability in reaction time (RT) performance was reliably correlated with the presence of at least one apolipoprotein E (APOE) ε4 allele and also to the levels of Aβ42 in the CSF in cognitively normal older adults (see also Rodrigue et al., 2012; Sperling et al., 2013). In contrast, however, numerous other studies have found no relationship between the biomarkers and concurrent cognitive performance (Aizenstein et al., 2008; Fagan et al., 2009; Jack et al., 2008; Nebes et al., 2013; Pike et al., 2011; Storandt et al., 2009; Vemuri, Wiste et al., 2009; Vemuri, Weigand et al., 2011).

Research regarding the role of APOE genotype on presymptomatic cognitive deficits has been similarly mixed. However, meta-analyses of the available literature suggest individuals with at least one ε4 allele exhibit small but reliable deficits in memory and executive function even among cognitively healthy participants (Small, Rosnick, Fratiglioni, & Bäckman, 2004; Wisdom, Callahan, & Hawkins, 2011). Once again, the mechanisms by which APOE influences cognitive performance have yet to be completely established. One potential mechanism may be increased brain amyloid deposition present in ε4 positive individuals (Morris et al., 2010; Reiman et al., 2009). Specifically, Morris et al. (2010) found that individuals with at least one APOE ε4 allele exhibited lower levels of Aβ42 and higher levels of PIB binding, but showed no difference in tau compared to individuals with no ε4 alleles. Thus, any differences in cognition between different APOE genotypes may be mediated by changes in Aβ42 or PIB.

There are a number of possible reasons why the relationships between AD biomarkers and cognitive performance remain elusive. First, there may be unmeasured protective factors that buffer against the cognitive effects of AD pathology such as cognitive reserve (e.g., Soldan et al., 2013; Stern, 2009). Alternatively, the standard psychometric tests that are part of many neuropsychological batteries simply may not be sensitive enough to be able to detect subtle presymptomatic cognitive deficits. Finally, as suggested by Storandt et al. (2012), there may be additional AD processes, either alone or in concert with presently studied biomarkers, which critically contribute to cognitive performance. In the present study, we aim to address these possibilities by performing casual and mediation analyses of the effects of AD biomarkers on a sensitive cognitive measure that combines tests of attentional control and semantic memory retrieval, two processes that are impaired in very early AD. We now turn to a discussion of the motivation of this specific task in greater detail.

Although episodic memory impairment is considered the hallmark cognitive characteristic of AD, there is accumulating evidence for a breakdown in attentional control systems as well (Balota & Faust, 2001; Faust & Balota, 2007; Perry & Hodges, 1999; Twamley, Ropacki, & Bondi, 2006). Attentional control can be conceived of, in part, as maintaining task goals throughout an experiment and is particularly stressed when multiple, competing levels of information must be inhibited or controlled. For example, one well known paradigm for measuring attentional control is the classic Stroop task (MacLeod, 1991; Stroop, 1935). In this task, participants are asked to name the color of the ink a color word is printed in (e.g., the word RED printed in blue ink), rather than the word itself. The prepotent "word" pathway competes with the task goal (naming the color) and must be controlled in order for the correct response to be made. Previous studies investigating Stroop performance in AD have shown intrusion errors to be particularly powerful in discriminating healthy aging from very mild AD dementia and in predicting later conversion (Balota et al., 2010; Hutchison, Balota, & Duchek, 2010; Spieler, Balota, & Faust, 1996).

Symptomatic AD individuals also exhibit poorer performance on tasks of semantic memory relative to cognitively healthy controls, particularly when demands on attention are maximized. For example, symptomatic AD participants score lower on tests of

category and verbal fluency (Canning, Leach, Stuss, Ngo, & Black, 2004; Hodges, Salmon, & Butters, 1992; Kirshner, Webb, & Kelly, 1984; Marczinski & Kertesz, 2006), in which individuals must not only generate exemplars but also track which items have already been produced to avoid repetitions. Poor performance on this task may in part result from the attentional demands of explicit retrieval and avoiding repetitions. Furthermore, symptomatic AD individuals show disrupted semantic priming under high attentional demands such as when the dominant meaning of a homograph must be inhibited (Balota & Duchek, 1991; Balota, Watson, Duchek, & Ferraro, 1999). Overall, these results indicate individuals with symptomatic AD are particularly affected when explicit semantic retrieval and/or attentional control is required.

Although there is evidence that memory measures may be a sensitive indicator of presymptomatic AD (see, e.g., Hedden, Oh, Younger, & Patel, 2013), there is also evidence that attention is a prerequisite for declarative memory (see, e.g., Jacoby, 1999). Furthermore, although standard neuropsychological tests are designed to target a single domain (e.g., episodic memory, executive function, etc.), any single test is unlikely to be process pure and indeed there is evidence to suggest changes in attentional control may contribute to episodic memory performance in early stage AD (e.g., Balota, Burgess, Cortese, & Adams, 2002; Tse, Balota, Moynan, Duchek, & Jacoby, 2010). While this does not negate the importance of episodic measures as indicators of AD risk, it does, however, lead to the possibility that directly targeting several cognitive domains within a single task may ultimately prove to be a more sensitive measure of AD pathology than a test of any "single" domain alone.

In addition to the neuropsychological literature, the present task is also motivated by results from functional neuroimaging. Indeed, while there is some debate regarding the neuroanatomical correlates of attention and semantic memory, it is generally agreed upon that attention tasks tap prefrontal structures (e.g., MacDonald, Cohen, Stenger, & Carter, 2000) while semantic processing relies more on medial temporal regions (e.g., Rogers et al., 2006). Interestingly, these same brain regions have been shown to exhibit elevated levels of PIB binding, even within cognitively healthy individuals (Mintun et al., 2006). For these reasons, we expect a task that taps both of these cognitive constructs to be consistent with the biological changes observed in AD.

Because of the observed changes in attentional control and explicit semantic memory retrieval, we developed a version of the category verification task (CVT) in which participants were presented with a category (e.g., "a unit of time") followed by a target item, and determined whether the target was a member of the given category (cf. Smith, Shoben, & Rips, 1974). For each category, two target exemplars within the category and two associatively related words that were not in the category were chosen. The strength of the category or associative relationship was varied across targets such that for each category, one exemplar and one foil were strongly related to the category and the other two were weakly related. For example, if the category is "a unit of time" the strong exemplar was "hour," the weak exemplar was "month," the strong associate was "clock," and the weak associate was "schedule." Importantly, using associatively related lures places strong demands on the attentional control system, similar to the Stroop task. Based on the sensitivity of error rates, as opposed to response latencies in past studies of Stroop performance (e.g., Balota et al., 2010; Duchek et al., 2013; Hutchison et al., 2010), one might a priori expect accuracy to be a particularly sensitive measure. Specifically, in order to say no to "clock" as a unit of time, one must control the strong association between "clock" and "time," and hence, breakdowns in attentional control may produce an increase in error rates in this condition.

In summary, the conflicting findings regarding biomarkers and cognitive performance suggest the relationship between biomarkers, cognition and risk of progression to AD is complex, and perhaps only the synergistic effects of multiple biomarkers have any measurable impact on cognition. We expect that the combination of explicit semantic retrieval and attentional control required in the CVT, particularly in the difficult associative condition, would be sensitive to AD-related cognitive changes. The present large dataset affords a unique opportunity to investigate the sensitivity of these measures to the buildup of biomarkers indicative of AD.

Goals of the Present Study

The goals of the present study are twofold. First, we investigated the effect of dementia status on the CVT as operationalized by the sensitive Clinical Dementia Rating Scale (CDR: Morris, 1993) by comparing a cognitively healthy older adult group to individuals diagnosed with the earliest detectable symptomatic stage of AD (CDR 0.5) using a 2 (Group) $\times$ 2 (Condition: Category or Associate) $\times$ 2 (Strength: Strong vs. Weak) mixed-effects ANOVA. This analysis examined whether the CVT is particularly sensitive to cognitive changes in individuals with clinically detectable symptoms of AD. In order to minimize age differences in this comparison, we only included cognitively healthy controls who were 65 or older in the analysis of AD status. Additionally, because category and associate items require different responses (i.e., "yes" vs. "no"), we also conducted follow-up analyses of the theoretically motivated Group $\times$ Strength interaction separately for each condition. The aim of these analyses was to establish the behavioral profile of clinically diagnosed AD on this novel task.

Our second, and primary, goal was to determine whether accumulating AD biomarkers in cognitively healthy controls would influence performance on the CVT in a similar manner as individuals with symptomatic AD. Thus, we employed a series of hierarchical regression and mediation models in our cognitively healthy control sample to examine the sensitivity of the CVT measures to accumulating AD biomarkers and, more importantly, to better understand the potential mechanisms by which they operate. It is important to emphasize that while the mediation analyses are based on correlations and thus cannot be equated to causality, it is possible to examine whether the pattern of correlations is consistent or inconsistent with a proposed model. Based on past findings (e.g., Storandt et al., 2012), the model examined here is one in which CSF tau and Aβ42 have unique and independent effects on cognition, which both operate primarily through elevated levels of PIB. In other words, the CSF markers both are related to elevated amyloid detected by PIB, which in turn is the primary cause of presymptomatic cognitive deficits. Thus, our second set of analyses addressed two primary questions: (a) Do the CSF biomarkers produce separate and independent detectable influence on CVT performance? and (b) Are these effects partially or completely mediated by PIB? A separate set of analyses addressed

the same questions regarding the effects of APOE and PIB, instead of the CSF biomarkers and PIB.

Method

Participants

A total of 372 individuals participated in this study: 305 cognitively healthy older adults and 67 individuals with very mild symptomatic AD (CDR 0.5), all recruited from the Charles F. and Joanne Knight Alzheimer's Disease Research Center at Washington University, as part of an ongoing research program on the longitudinal progression of AD. All data in the present report are from participants' first time completing this particular cognitive task (i.e., the CVT). All individuals were assessed for dementia using the Washington University CDR scale (Morris, 1993). The CDR assigns a rating of 0, 0.5, 1, 2, or 3 reflecting no, very mild, mild, moderate, or severe dementia, respectively. The CDR is based on a 90-min clinical interview with the participant and independently with an observant informant and is made without reference to performance on psychometric testing. The accuracy of this research group in diagnosing even very mild dementia (CDR 0.5) has been very high (93% accurate), as confirmed by later autopsy in past studies (Berg et al., 1998; Storandt, Grant, Miller, & Morris, 2006). Because the present study focused on the earliest stages of symptomatic AD, we only included individuals who were given a CDR rating of either 0 or 0.5.

The term symptomatic AD is intended to encompass the entire clinical spectrum of the disease (cf. Morris et al., 2014); therefore, we include the descriptor "very mild" to indicate these are individuals in the earliest detectable stages of the disease (i.e., CDR 0.5). It is possible that some of these participants had mild cognitive impairment rather than true Alzheimer dementia. Furthermore, five CDR 0 participants and three CDR 0.5 participants were later determined to have symptoms of dementia not due to AD, for example, frontotemporal dementia. Because we were interested in Alzheimer dementia specifically, these eight participants were excluded from all further analysis.[1]

Psychometric Testing

All participants were administered a standard psychometric test battery by an examiner who was blind to the individual's CDR rating. However, depending upon the individual's age upon entry into the study, one of two batteries was administered, and because of the differences across batteries, in the present report, we only examine the standard tests that both batteries have in common. These tests included the Mini Mental State Examination (MMSE: Folstein, Folstein, & McHugh, 1975), a test of working memory using the Letter Number Sequencing subtest of the Wechsler Adult Intelligence Scale (Wechsler, 1997), and a test of episodic memory using the Selective Reminding Test (Grober, Buschke, Crystal, Bang, & Dresner, 1988). The measure of interest from the Selective Reminding Test was the number of correct words freely recalled across all trials. Visual-perceptual motor performance was assessed using Trail Making A (Armitage, 1946), and Animals Naming (Goodglass & Kaplan, 1983) was administered as a measure of lexical-semantic retrieval. Although delayed memory measures tend to be quite sensitive to CDR status, due to the differences across batteries we did not have a common delayed measure available for all participants, and thus, delayed memory was not examined here.

A series of *t* tests were conducted comparing test performance between our age-matched control sample (CDR 0 with ages 65 years and greater) and the CDR 0.5 sample. Results confirmed that the CDR 0.5 group performed worse on all tests ($ps < .05$). Table 1 provides demographics and the results from the psychometric tests. It is important to note that the CDR 0.5 participants have relatively high MMSE scores of 27.6, which, although significantly lower, are quite close to the CDR 0 MMSE scores of 28.8. Thus, these CDR 0.5 individuals are indeed in the very earliest detectable stages of symptomatic AD.

APOE Genotyping, CSF Measurement, and PET PIB Imaging

Our primary goal in the present study was to evaluate the relationship of well-established biomarkers on cognitive performance as measured with the CVT. Thus, we selected a subsample of our cognitively healthy control subjects who (a) had APOE genotype information available and (b) had CSF measurements, PIB imaging and cognitive testing within 3 years of each other. We selected 3 years as the cutoff point because the cognitive battery that includes the CVT is administered once every 3 years. However, the average length between measurements of these variables was 78 days.[2] Additionally, in order to avoid spurious effects due to multivariate outliers, we first calculated Mahalanobis D^2 for each individual in the subsample and eliminated any observation that occurred with a probability of less than .001. The procedure eliminated three participants. Thus, all analyses involving the biomarkers were conducted on this subsample of 113 cognitively healthy controls. Table 2 lists the full demographics of this sample, and as shown, this sample is quite representative of the larger group of CDR 0 participants who completed the CVT. We now turn to a brief description of the assessment methods for the primary biomarkers of interest. A fuller description is given in the associated references.

APOE genotyping was performed using standard procedures with TaqMan assays (Applied Biosystems, Foster City, CA) for both rs429358 (ABI#C_3084793_20) and rs7412 (ABI#C_904973_10). Due to the small number of individuals for some of the allele groups, ($\varepsilon22 = 1$, $\varepsilon23 = 10$, $\varepsilon24 = 3$, $\varepsilon33 = 62$, $\varepsilon34 = 31$, and $\varepsilon44 = 6$), we collapsed the groups into presence of at least one ε4 allele (ε4+, $n = 40$) or absence of any ε4 alleles (ε4–, $n = 73$).

[1] One may be concerned that cerebrovascular mechanisms may also contribute to group differences observed here. However, our participants are well screened on intake and assessed for history of heart attack, atrial fibrillation, angioplasty, cardiac bypass surgery, pacemaker, and congestive heart failure. We correlated levels of biomarkers with the presence of a recent cardiac event and no correlations were significant. That is not to say cerebrovascular mechanisms are not in play here, but at least in the current sample they are likely minimized due to our screening procedures.

[2] Due to a calculation error, one participant who had an interval between PIB imaging and CVT testing that was 2 months past the cutpoint was included. Reanalysis of all the models with this subject excluded did not change any of the obtained results.

Table 1
Psychometric Test Performance of Cognitively Healthy Controls and Individuals With Symptomatic Alzheimer's Disease

Variable/test	All controls			Controls age-matched to the CDR 0.5 participants			CDR 0.5		
	M	*n*	*SD*	*M*	*n*	*SD*	*M*	*n*	*SD*
Age (years)	68.3	299	9.0	73.2	204	5.9	74.3	64	7.1
Education (years)	15.7	299	2.5	15.3	204	2.5	14.8	64	2.9
MMSE	29.0	299	1.3	28.8	204	1.4	27.6	64	2.5
Animal Fluency	21.2	299	5.9	20.2	204	5.6	16.4	64	6.1
Trail Making A	33.7	299	12.7	36.4	204	13.1	45.6	64	25.4
Letter Number Sequencing	10.1	276	2.7	9.4	183	2.5	8.1	54	3.0
Selective Reminding	31.0	297	5.6	30.0	202	5.5	22.2	61	9.6

Note. The range of possible scores for each test is as follows: Animal Fluency: 0 and above; Trail Making A: 0–180 s; Letter Number Sequencing: 0–21; Selective Reminding: 0–48. For all tests, a higher score indicates better performance with the exception of Trail Making A, for which a higher score indicates poorer performance. "All controls" refers to our entire sample of CDR 0 participants and the "age-matched controls" are the participants used in the comparison to the CDR 0.5 individuals. CDR = Clinical Dementia Rating Scale; MMSE = Mini Mental State Examination.

CSF was collected and analyzed in accord with a standard protocol (e.g., Fagan et al., 2007). Briefly, CSF samples (20–30 mL) were collected after an overnight fasting period and then aliquoted (500 μL) into polypropylene tubes and stored at −84°C. Analyses were conducted on these samples after a single thaw using standard, commercial enzyme-linked immunosorbent assay (INNOTEST, Innogenetics, Ghent, Belgium). The reliability of CSF measurements was recently assessed using data from 40 independent laboratories worldwide (Mattsson et al., 2011). Although there is considerable intercenter variability in CSF measurements (16–28% for INNOTEST), it is more important to understand the within-laboratory variability, as all measurements in the present study were analyzed at Washington University. Reference laboratories that are accustomed to processing large numbers of CSF samples analyzed the same samples on six different plates and the coefficient of variation was calculated as a measure of within laboratory consistency (e.g., smaller coefficient of variation means more consistent measurements). Within-laboratory coefficients of variation for the three CSF markers ranged from 3.2% to 24%. While this indicates substantial variability even within a single laboratory, it is important to point out that reduced reliability of the CSF markers would only make it more difficult to obtain the correlations we present in our analyses that follow. Quality control and reliability studies are ongoing and the precision of CSF analyses will continue to increase as sources of variability are identified and eliminated (Mattsson et al., 2011).

Table 2
Participant Demographic Characteristics and Biomarker Levels of the 113 Participants Used for the Mediation Analyses

Variable	*M*	*SD*
Age	65.2 years	8.2
Education	15.7 years	2.6
MMSE	29.2	1.1
Tau	275 pg/ml	162
Aβ42	652 pg/ml	253
PET-PIB	.338	.46
LP-PIB[a]	87 days	380
LP-CVT[b]	117 days	342
PIB-CVT[c]	30 days	382

Note. MMSE = Mini Mental State Examination; Aβ42 = amyloid-beta 42; PET = positron emission tomography; PIB = Pittsburgh compound-B; LP = lumbar puncture; CVT = category verification task.
[a] The difference between time of cerebrospinal fluid (CSF) assessment and PIB scan. [b] The difference between time of CSF assessment and CVT testing. [c] The difference between time of PIB scan and CVT testing.

Fibrillar amyloid imaging was conducted via PET with PIB as fully described elsewhere (e.g., Mintun et al., 2006). Regions of interest were constructed based on individual's MRI scan, and a binding potential for each region of interest was calculated (Logan et al., 1996). The binding potentials from several regions known to have high PIB uptake in AD were averaged to create a single, mean cortical binding potential. This measure was formed from the average of the precuneus, prefrontal cortex, gyrus rectus and lateral temporal regions. Test–retest reliability of the mean cortical binding potential has been shown to be remarkably stable with a change of only .25% between two consecutive scans and an intraclass correlation of 1.0 (Su et al., 2013).

Category Verification Task

Forty categories and their respective exemplars and associates were selected to be used as stimuli in the CVT. As described previously, for each category, two target exemplars (Category condition) and two associatively related foils (Associative condition) were chosen, to generate a total of 160 category-target pairs. Half of the items were strongly related to the category, and half were weakly related. The exemplar items were based on the Van Overschelde, Rawson, and Dunlosky (2004) category norms and the associate items were selected using the Nelson, McEvoy, and Schreiber (2004) free-association norms. A full list of materials is available from the authors upon request.

On each trial, the following events occurred: (a) a fixation cross was displayed in the center of the screen for 1,000 ms, (b) the category label was displayed for 2,000 ms, (c) a blank screen was displayed for 650 ms, and (d) the target item was displayed until a response was made. Participants were instructed to use the index finger of their right hand to press the P key if the target was a member of the category and the index finger of their left hand to press Q if the target was not a member of the category. Labels were posted above each of the response keys as a reminder of which key corresponded to "yes" or "no." We did not reverse the key map-

pings for left-handed individuals, therefore to be sure handedness was not confounded with group status, we conducted a Pearson chi-square test comparing the proportion of right-handed individuals to the proportion of left-handed or "unknown" participants. It is important to note that handedness did not differ between groups (CDR 0 = 87% right-handed; CDR 0.5 = 84% right-handed, p = .43). The order of presentation of categories and targets was randomized anew for each individual. Each session began with six practice trials during which feedback was provided after incorrect responses only. Feedback was not provided during test trials. Participants were encouraged to respond as quickly and as accurately as possible.

Results

In pursuit of our first goal, we first compared CVT performance between the CDR 0.5 participants and an age-matched sample of cognitively healthy controls. To minimize the influence of outliers, individual data were screened in the following way. First, all response latencies faster than 200 ms and slower than 7,000 ms were removed, and of the remaining latencies, trials that exceeded three standard deviations from the individual mean were also removed. This procedure resulted in the removal 3% of the trials for the cognitively healthy controls and 4% of the trials for the symptomatic AD groups. We chose an upper cutoff of 7,000 ms based on visual inspection of group histograms. We selected a three-standard-deviation individual cutoff to strike a balance between the need to eliminate extreme outliers that could unduly influence the mean performance and the desire to retain as much data as possible. Although the symptomatic AD group had significantly more trials excluded from analysis, this procedure was necessary to avoid the undue influence of a very few extremely long RTs.[3] In addition, one healthy older adult had an extremely high number of errors (over 90%, which was likely due to not having the key mapping correct) and was excluded from all subsequent analyses.

In the following analyses of response latencies,[4] we present analyses on *z*-transformed data, which controls for group related differences in general slowing (see Faust, Balota, Spieler, & Ferraro, 1999) The *z*-scored RTs are formed by standardizing individual, trial-level RTs to each participant's overall mean and standard deviation. The *z*-scored condition means are then submitted to analysis of variance.

Z-Score Analyses

Figure 1 displays the mean RT data, and Figure 2 displays the mean *z* scores as a function of group and condition. The ANOVA on the *z* scores (which controls for overall response latencies) yielded a significant main effect of group, $F(1, 266) = 19.60$, $p < .001$, $\eta_p^2 = .07$, condition, $F(1, 266) = 136.92$, $p < .001$, $\eta_p^2 = .34$, and strength, $F(1, 266) = 219.18$, $p < .001$, $\eta_p^2 = .45$. There were two reliable interactions. Specifically, as predicted, the Group × Condition interaction was reliable, $F(1, 266) = 5.59$, $p = .019$, $\eta_p^2 = .02$, which indicated that the CDR 0 individuals produced a smaller effect of exemplar versus associate ($z = .23$) than the CDR 0.5 individuals ($z = .36$). In addition, there was a significant two-way interaction between condition and strength that reflected a cross-over effect of strength for associate items ($z = -.19$) compared to category items ($z = .55$), $F(1, 266) = 949.35$, $p < .001$, $\eta_p^2 = .78$. The three-way interaction was not significant, $F(1, 266) = .18$, $p = .674$, $\eta_p^2 = .001$.

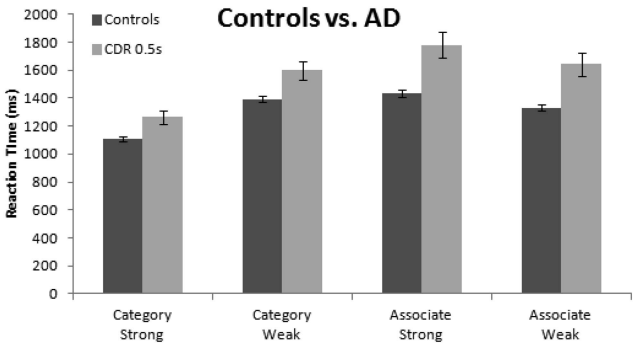

Figure 1. Mean reaction times as a function of group and condition. Error bars represent the standard error of the mean. AD = Alzheimer's disease; CDR = Clinical Dementia Rating Scale.

Accuracy Analyses

The percent correct as a function of group and condition are displayed in Figure 3. The ANOVA revealed reliable main effects of group, $F(1, 266) = 36.49$, $p < .001$, $\eta_p^2 = .12$, condition, $F(1, 266) = 27.37$, $p < .001$, $\eta_p^2 = .09$, and strength, $F(1, 266) = 20.71$, $p < .001$, $\eta_p^2 = .07$. The Group × Condition interaction was again highly reliable, $F(1, 266) = 18.79$, $p < .001$, $\eta_p^2 = .07$, which indicated that the cognitively healthy adults produced a much smaller effect of condition ($M = 0.78$) than the CDR 0.5 individuals ($M = 8.29$). The Group × Strength interaction was also significant, $F(1, 266) = 4.83$, $p = .029$, $\eta_p^2 = .018$, as was the Condition × Strength interaction, $F(1, 266) = 587.45$, $p < .001$, $\eta_p^2 = .69$. The Group × Condition × Strength interaction also approached significance, $F(1, 266) = 3.04$, $p = .082$, $\eta_p^2 = .01$.

Planned follow-up analyses indicated that the Group × Strength interaction for the category items was not significant, $F(1, 266) = .05$, $p = .816$, $\eta_p^2 = 0$, whereas this interaction was reliable in the associate condition, $F(1, 266) = 6.82$, $p = .01$, $\eta_p^2 = .03$. As predicted, and as shown in Figure 3, the symptomatic AD participants had difficulty in responding correctly to (i.e., rejecting) the strong associate items.

The results from these analyses are quite clear. After controlling for group differences in processing speed, the *z*-score analyses indicated that there was a larger effect of exemplar versus associ-

[3] We also used a more conservative trimming procedure where no upper screen was used. This resulted in equal numbers of trimmed RTs for each group (less than 2%). Results did not change when this procedure was used. Furthermore, we used a 2-*SD*s cutoff rather than 3 *SD*s, which resulted in substantially more trials being excluded. Again, results were qualitatively identical and if anything, became more robust.

[4] We have a substantially greater number of cognitively normal controls than individuals with very mild AD dementia, which possibly could be influencing the statistical tests. Thus, we also conducted a bootstrap analysis of the critical comparisons including the three-way interactions, the effect of item strength within each condition, and the Group × Condition interaction for the accuracy, RT and *z* -RT measures. Each bootstrap was based on 100,000 replications, and the results were the same as the standard analyses.

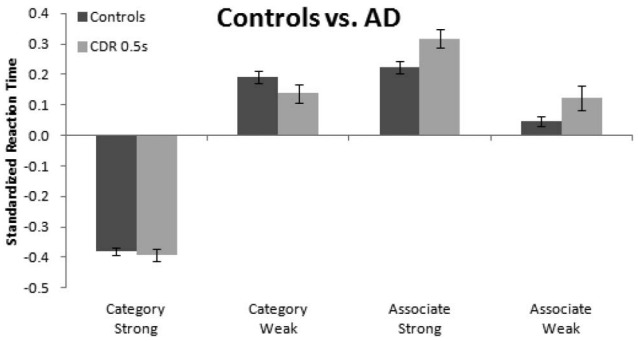

Figure 2. Mean *z*-scored reaction times as a function of group and condition. Error bars represent the standard error of the mean. AD = Alzheimer's disease; CDR = Clinical Dementia Rating Scale.

ate condition for the CDR 0.5 group than the control group, precisely as one would expect. Although this effect was reliable, it was small.[5] The effect in accuracy was much clearer. Specifically, the results from the accuracy analyses indicated the very mild AD individuals had particular difficulty in overcoming the familiarity signal from the associate items, which was particularly strong for the high-associate items, reflected by the increased likelihood to incorrectly respond "yes" to the items that had high overlap (e.g., incorrectly indicate that "Clock" is a "Unit of Time"). These results are consistent with accumulating evidence indicating that accuracy measures may be more sensitive than response latency measures, after controlling for overall processing speed differences via *z*-score transformations (see Balota et al., 2010; Duchek et al., 2013; Hutchison et al., 2010; Spieler et al., 1996).

More importantly, these analyses establish that accuracy in the associate condition appears to be particularly sensitive to CDR status. To further verify the utility of the CVT in discriminating cognitively healthy controls from individuals with symptomatic AD, we conducted a series of logistic regressions predicting CDR status from age, education, and a standard psychometric test (MMSE, Animal Fluency, Trail Making A, Letter Number Sequencing, or Selective Reminding) in the first step, followed by the overall accuracy in the associate condition in the second step. The CVT measure accounted for significant variance above and beyond each of the tests ($ps < .05$, R^2 change = .06 to .08) with the exception of Selective Reminding ($p = .12$, R^2 change = .01). These regressions suggest that the CVT can predict CDR status above and beyond many psychometric tests. However, the differences in the number of participants who completed each test (see Table 1) make rigorous comparisons difficult. Moreover, the primary goal of these analyses was not to determine the power of the CVT in predicting CDR status but rather to understand how the CVT behaves in a group with clinically detectable symptoms of AD (e.g., CDR status of 0.5).

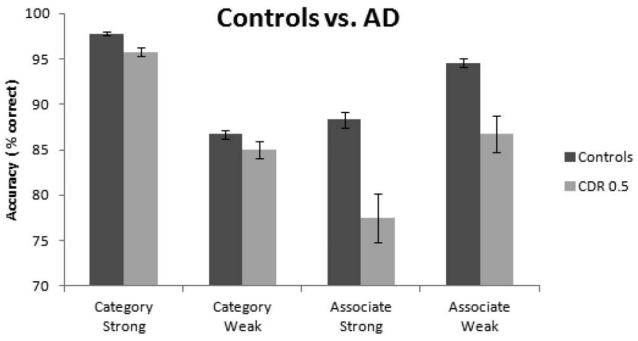

Figure 3. Mean percent correct as a function of group and condition. Error bars represent the standard error of the mean. AD = Alzheimer's disease; CDR = Clinical Dementia Rating Scale.

Table 3
Partial Correlations Between the CSF, PIB, and Genetic Markers After Controlling for Age and Education

	P-tau	Aβ42	PIB	APOE
Tau	.74**	.06	.50**	.09
P-tau		.12	.39**	.08
Aβ42			−.47**	−.31**
PIB				.24*

Note. CSF = cerebrospinal fluid; PIB = Pittsburgh compound-B; Aβ42 = amyloid-beta 42; APOE = apolipoprotein E.
* $p < .05$. ** $p < .01$.

AD Biomarkers and CVT Performance

We now turn to the primary goal of the present study and model the relationships of tau, p-tau$_{181}$, Aβ42, and PIB on performance in the CVT in our subsample of 113 cognitively healthy controls. Because both tau and p-tau$_{181}$ are positively skewed, we applied the natural log transformation to better approximate normality. All variables of interest, including CVT measures and the biomarkers, were then standardized within these 113 participants. We first present the correlation among the biomarkers in Table 3. Importantly, replicating past findings (e.g., Storandt et al., 2012), PIB was positively correlated with tau and negatively correlated with Aβ42, while the two CSF measures were uncorrelated with one another.

More importantly, we turn now to the relationships between the biomarkers and the CVT measures. Our modeling strategy proceeded in several steps. First, to establish the basic relationships among the variables of interest, we conducted a series of Pearson product–moment correlations between the biomarkers (tau, p-tau, Aβ42, PIB, and APOE), and each of the measures from the CVT (RT and accuracy) after controlling for the effects of age and education. These correlations are presented in Table 4. The standardized residual scatter plots of these measures for the accuracy on associate trials are presented in Figure 4.

There are several observations to note in Table 4. First, accuracy was robustly correlated with PIB, and to a much lesser extent,

[5] Following Tse et al., 2010, we conducted ex-Gaussian analyses on these data as research has shown the slow tail of the RT distribution to be highly sensitive to AD related cognitive changes. However, contrary to the results of Tse et al., we did not find the effect of AD to be isolated to the slow tail, but instead manifested across the entire distribution. This suggests a fundamentally different mechanism is operating in the current CVT compared to standard attentional control tasks. Given the differences between the present CVT and the tasks used in Tse et al., we focus on the more standard analyses of accuracy and mean RTs in the present report.

Table 4
Partial Correlations Between the CVT Measures and the Biomarkers Controlling for Age and Education

	Tau	P-tau	Aβ42	PIB	APOE
Category accuracy	−.09	−.12	0	−.09	−.16
Associate Accuracy	−.20*	−.21*	.22*	−.44**	−.20*
Category RT	.04	.12	.07	.14	.04
Associate RT	.12	.16	−.09	.33**	.07

Note. CVT = category verification task; Aβ42 = amyloid-beta 42; PIB = Pittsburgh compound-B; APOE = apolipoprotein E; RT = reaction time.
* $p < .05$. ** $p < .01$.

Aβ42 and tau. Specifically, accuracy increased with increasing levels of Aβ42, and also decreased with increasing levels of tau, p-tau, and PIB binding, which is exactly the pattern one would expect based on how these biomarkers accumulate in individuals at risk for developing AD (Fagan et al., 2007). Second, the response latencies to the more difficult associate condition were also sensitive to levels of PIB. Third, none of the measures from the category condition, which does not demand the same degree of attentional control as the associate items, correlated with the CSF markers. Overall, this dissociation between the category and associate measures in regard to sensitivity to the biomarkers is striking and suggests that the high level of attentional control that is required to successfully reject the associatively related words can differentiate individuals who are at increased risk for developing symptomatic AD. These preliminary analyses, along with the evidence from the CDR 0 versus CDR 0.5 comparisons earlier, indicate that the accuracy to the associate condition of the CVT is sensitive to multiple biomarkers of the underlying processes of AD pathology.

We now turn to a series of hierarchical regression analyses to examine how much independent variance in CVT accuracy was accounted for by each of the biomarkers. Based on our initial correlations, we used accuracy to the associate trials as the dependent variable and entered each of the following predictors in separate steps: age, education, tau, Aβ42, and the Tau × Aβ42 interaction. The R^2 change due to each additional variable in the model, and the beta weights are presented in Table 5. The R^2 change was significant at each step ($ps < .05$), indicating each variable accounted for unique variance in accuracy above and beyond the preceding variables. It is interesting to note that both age and education had a significant influence on CVT performance above and beyond the CSF biomarkers. More importantly, the CSF biomarkers produced a reliable interaction. The graph of this interaction is presented in Figure 5 and as can be seen, accuracy was particularly impaired when abnormal levels of both CSF markers are present.

As noted in the introduction, Storandt et al. (2012) demonstrated tau and Aβ42 interact to predict levels of PIB binding. The next question we addressed is whether the cognitive effects of the CSF markers are mediated by their joint influence on PIB. If PIB does not significantly mediate the relationship, then one would have evidence for an additional process of presymptomatic AD-related cognitive changes that is unrelated to amyloid deposition reflected by PIB. As such, we performed a mediation analysis using the SPSS macro PROCESS developed by Hayes (2013), with accuracy as the dependent variable, tau, Aβ42 and their interaction as predictors, PIB as the proposed mediator, and age and education as covariates. The results of this model are presented graphically in Figure 6. As shown (and replicating the relationship among biomarkers in Storandt et al., 2012), tau, Aβ42, and their interaction account for significant variance in levels of PIB, and PIB itself accounted for significant variance in CVT accuracy in the final model. Most importantly, PIB completely mediated the relationship of tau (indirect effect = −.15, $p < .05$) and Aβ42 (indirect effect = .13, $p < .05$) on accuracy. Indeed, after accounting for the indirect effect through PIB, the direct effects of these CSF biomarkers on accuracy were both no longer significant, indicating total mediation.

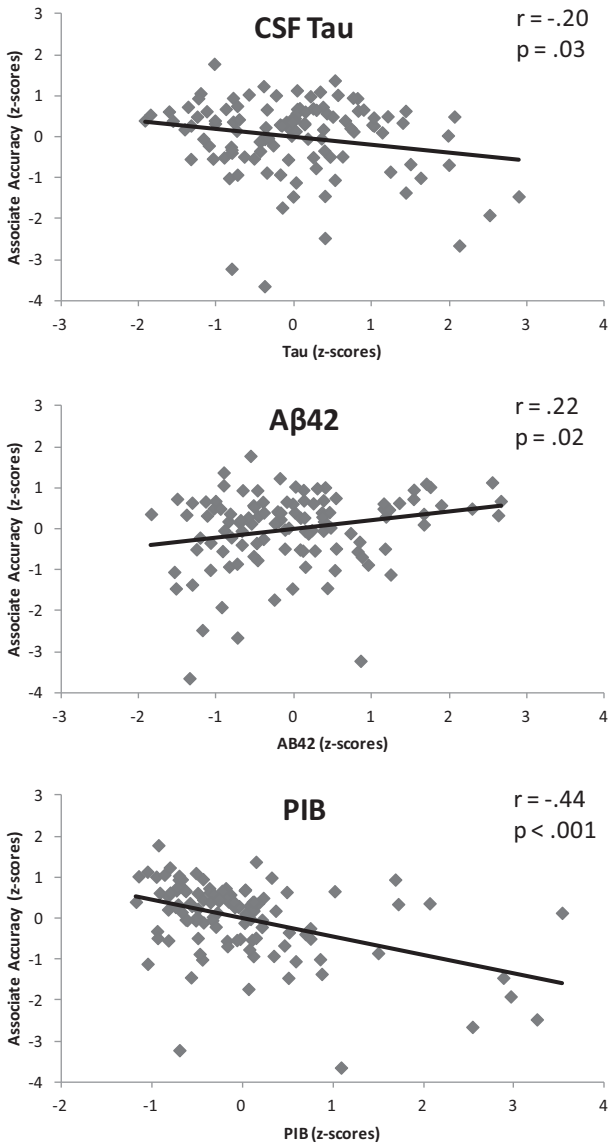

Figure 4. Standardized residual plots relating category verification task accuracy to the biomarkers after controlling for age and education. CSF = cerebrospinal fluid; Aβ42 = marker of amyloid deposition; PIB = Pittsburgh compound-B.

Table 5
Results of the Regression Model Relating the CSF Biomarkers to CVT Performance

Step	Predictor added	R^2 change	*p* value[a]	Beta weight[b]
1	Age	.112	<.001	−.203
2	Education	.055	.008	.206
3	Tau	.035	.032	−.116
4	Aβ42	.047	.011	.111
5	Tau by Aβ42	.063	.002	.266

Note. CSF = cerebrospinal fluid; CVT = category verification task; Aβ42 = marker of amyloid deposition.
[a] Significance of the increment in R^2. [b] Beta weight of the predictor in the final model (Step 5).

Interestingly, while the Tau × Aβ42 interaction was also partially mediated by PIB as one would expect (indirect effect = .08, $p < .05$), the direct effect of the interaction was still significant ($\beta = .19$, $p = .03$). This indicates that although the individual effects of tau and Aβ42 on cognitive performance do not account for variance above that accounted for by PIB, when tau and Aβ42 are both "abnormal," there may be an additional process related to presymptomatic cognitive changes that is not accounted for by levels of fibrillar amyloid.[6] In addition, education had a unique influence on CVT accuracy above and beyond all other variables in the model. Although we did not test for whether education moderated the influence of the biomarkers on cognitive performance, the fact that it still accounted for unique variance suggests this is an important variable to consider in future models, possibly as a marker of cognitive reserve (e.g., Stern, 2009).

Finally, turning to the effects of APOE, we tested a separate mediation model in which the effect of APOE genotype was treated as the focal predictor, accuracy was the outcome, tau, Aβ42, and PIB were potential mediators and age and education were treated as covariates. The results of this model are presented graphically in Figure 7. Again, there are several important points to note here. First, replicating Morris et al. (2010), APOE was significantly related to Aβ42 ($\beta = -.65$, $p < .001$) but not to tau ($\beta = .18$, $p = .34$). Furthermore, the only significant mediation occurring in this model was the pathway from APOE to Aβ42 to PIB to accuracy. There was no significant path from APOE directly to PIB after controlling for Aβ42 ($\beta = .09$, $p = .52$). Critically, to our knowledge, this is the first demonstration that the influence of APOE on cognition operates primarily through Aβ42 and subsequently through PIB.

The present results suggest that associate accuracy from the CVT may be particularly sensitive to the accumulation of underlying pathology indicative of AD. As noted, the relationship between standard psychometric measures and AD biomarkers has been mixed. In order to further explore the specificity of associate accuracy to the biomarkers, we repeated our original mediation model but also included a standard psychometric test (e.g., Animal Fluency, Trail Making A, Letter Number Sequencing, or Selective Reminding) as an additional covariate. All the predictors (tau, Aβ42, and their interaction) and proposed mediator (PIB) remained the same. Importantly, results indicated the CVT was sensitive to the interaction of the CSF measures as well as to PIB above and beyond each of the four tests (all *p*s $< .05$). Furthermore, PIB still significantly mediated the relationship between the CVT and each of the CSF biomarkers and their interaction (indirect effect *p*s $< .05$) with only one exception. Specifically, when Selective Reminding was used as a covariate, the indirect effects through PIB were only marginally significant in the predicted direction (*p*s $< .1$).

Collectively, these results indicate the CVT is sensitive to underlying pathology of AD above and beyond a set of standard psychometric tests. In this light, it is interesting to note that in a recent study of autosomal dominant AD, performance in this same task was able to discriminate between carriers and noncarriers of genetic mutations in cognitively healthy CDR 0 individuals (Storandt, Balota, Aschenbrenner, & Morris, 2014). This provides converging evidence that the CVT is a sensitive measure of underlying biological changes preceding the onset of symptomatic AD in a particularly relevant cohort.

General Discussion

The purpose of the present research was to provide an examination of the combined influence of semantic retrieval and attentional control in discriminating cognitively healthy aging from symptomatic AD, and more importantly to examine if this measure is sensitive to accumulating AD-related biomarkers in healthy control individuals. We discuss each of these issues in turn.

CVT Performance in Early Stage AD

A necessary first step in this research program was to establish that the task is indeed sensitive to early stage AD. We expected this to be the case because it is clear that very mild symptomatic AD participants are impaired on explicit semantic retrieval tasks (Canning et al., 2004; Hodges et al., 1992; Kirshner et al., 1984; Marczinski & Kertesz, 2006) and on tasks that place a high demand on attentional control systems (see reviews by Balota & Faust, 2001; Faust & Balota, 2007). In the present study, we used a version of the CVT that places a high demand on both explicit semantic retrieval processes and attentional control. As expected, the accuracy to the associate foils decreased in very mild symptomatic AD, suggesting a breakdown in the ability to control the associative information in order to produce a correct "no" response. These results are consistent with recent arguments that symptomatic AD individuals are more likely to allow inappropriate but prepotent stimulus dimensions to drive their responses rather than being able to take additional processing time to be able to produce the correct response (e.g., Duchek et al., 2013; Hutchison et al., 2010). Specifically, the symptomatic AD participants in the present study may have allowed the prepotent familiarity signal from the associative information to drive their response thereby producing an increase in error rates, especially for the high associatively related items (e.g., Unit of Time: "Clock").

[6] There is an additional point to note in Figure 6. Specifically, the effect of increased age on the CVT appears to operate through PIB as the beta weight for age on CVT was no longer significant after PIB was included in the model. However, this should be interpreted with caution as a strict mediation test was not undertaken here. Indeed, other studies have shown age influences PIB through its effect on the CSF biomarkers (Storandt et al., 2012). Our goal in the present analysis was not a definitive investigation of how increasing age affects cognitive performance but rather to understand the effects of the biomarkers while partialing out any variance due to age; therefore, we did not undertake a mediation analysis of age.

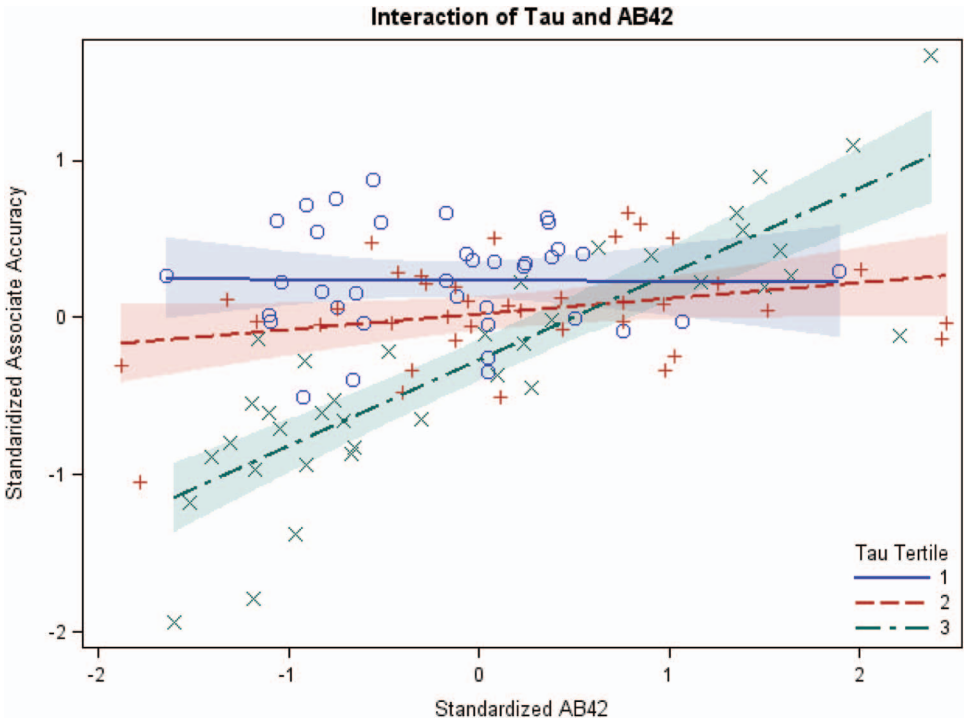

Figure 5. Interaction between cerebrospinal fluid tau and Aβ42 on associate accuracy. Lines represent simple slopes of Aβ42 at tertiles of tau. Aβ42 = marker of amyloid deposition. See the online article for the color version of this figure.

Sensitivity to Biomarkers in Healthy Controls

The critical finding in the present study is that the accuracy in the attention demanding associate condition was also correlated with AD biomarkers in a large, well-characterized sample of cognitively normal control individuals. It is noteworthy that these relationships were only reliable in the associate measures and not in the measures from the category condition. This pattern suggests that attentional control mechanisms in semantic memory retrieval may be a sensitive marker of underlying AD pathology. Importantly, the accuracy in the associate condition was a particularly powerful indicator of AD risk and correlated significantly with all four biomarkers analyzed here, above and beyond several standard psychometric tests that were available for this sample.

More importantly, our primary goal in the present research was to determine whether the well-studied biomarkers of AD impair cognition in a manner consistent with a model that proposes multiple processes that jointly operate through increased amyloid burden. Importantly, we extended the results of Storandt et al. (2012) by showing both tau and Aβ42 account for unique variance in a cognitive measure (CVT accuracy) and when abnormal levels of both biomarkers are present the effects on cognition are multiplicative. Previous studies of CSF biomarkers have indicated the importance of the tau-to-Aβ42 ratio

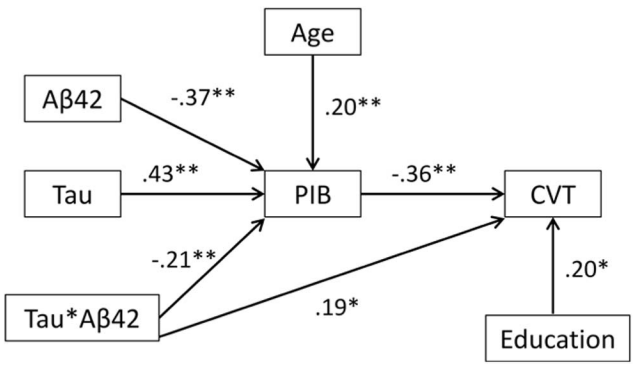

Figure 6. Final model depicting the relationships between AD biomarkers and CVT performance. Aβ42 = amyloid-beta 42; PIB = Pittsburgh compound-B; CVT = category verification task. * $p < .05$. ** $p < .01$.

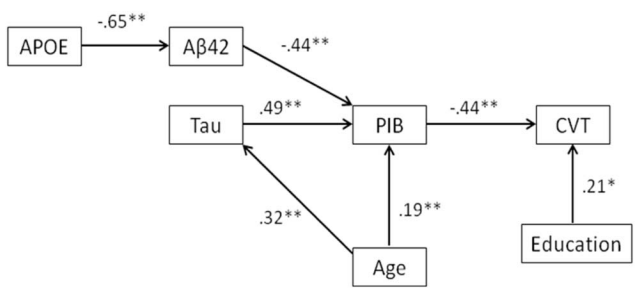

Figure 7. Model showing the relationship of APOE on CVT performance is mediated by Aβ42 and PIB. APOE = apolipoprotein E; Aβ42 = amyloid-beta 42; PIB = Pittsburgh compound-B; CVT = category verification task. * $p < .05$. ** $p < .01$.

in predicting future progression to symptomatic AD (e.g., Fagan et al., 2007) and in showing correlations with concurrent cognitive performance (e.g., Duchek et al., 2009). Critically, we demonstrated there is a synergistic effect of CSF biomarkers on a cognitive outcome by using regression analyses. Importantly, these analyses indicate the CVT is sensitive to both processes of AD that these biomarkers are thought to reflect.

Furthermore, PIB totally mediated the main effects of the CSF biomarkers on CVT performance, which indicates the influence of isolated CSF biomarker accumulation cannot be separated from that due to PIB, at least in the present data. This pattern nicely extends recent arguments by Storandt et al. (2012) regarding the relation among the biomarkers to a cognitive measure sensitive to AD. However, it is also noteworthy PIB only *partially* mediated this effect because there was an independent *interaction* of the two CSF markers (see Figure 5). Thus, the joint effect of the CSF biomarkers does appear to have an effect above and beyond simply increasing PIB binding.

Turning to the effect of APOE on cognition, several studies have detected small but consistent differences on cognitive measures as a function of the number of ε4 alleles (e.g., Small et al., 2004; Wisdom et al., 2011). However, the mechanism by which APOE influences cognitive performance has not been well established. In the present study, we were able to not only show an effect of APOE genotype on performance in the CVT, but also that the effect was entirely mediated by the pathway from Aβ42 to PIB. This finding is important because it provides evidence for a mechanism by which APOE negatively impairs cognition (e.g., by increased amyloid burden). Future research into the cognitive consequences of APOE will also need to take into consideration the levels of Aβ42.

Overall, our results are consistent with a model in which tau and Aβ42 are related to increased amyloid burden measured with PIB, and importantly, high amyloid burden has negative consequences on a cognitive task. However, the remaining significant interaction of the CSF markers alludes to the possibility of a second process that is unrelated to PIB and only occurs when levels of both CSF markers are abnormal.

As noted previously, investigations of AD biomarkers and cognitive performance have yielded mixed results, with some studies showing no relationship (e.g., Aizenstein et al., 2008; Fagan et al., 2007; Jack et al., 2008; Nebes et al., 2013; Pike et al., 2011; Storandt et al., 2009; Vemuri, Wiste et al., 2009; Vemuri, Weigand et al., 2011). At present, it is unclear whether many of the past cognitive measures simply lack the necessary sensitivity to detect prodromal changes in cognitive performance due to biomarkers or if cognitive changes in the previous measures only manifest after the disease process has progressed significantly. Perhaps both amyloid deposition and neuronal degeneration must occur before more widespread cognitive changes can be detected. Given our results, it is clear that the relationship between AD biomarkers and cognitive performance is nuanced and provides an important avenue of further research. Future studies should investigate potential mediators of the chain of events from biomarker accumulation to cognitive deficits. For example, there is evidence that in a group that includes some individuals with mild cognitive impairment, the effect of PIB on episodic memory measures appears to be mediated by changes in hippocampal volume (Mormino et al., 2009).

Conclusions

The present study adds to the growing body of research implicating a key role of attentional control mechanisms in early stage symptomatic AD (Balota et al., 2010; Hutchison et al., 2010; Perry & Hodges, 1999; Tse, Balota, Yap, Duchek, & McCabe, 2010; Twamley et al., 2006). Moreover, we have provided evidence that the combination of attentional control with a semantic retrieval task is particularly sensitive in discriminating not only healthy controls from individuals with very mild symptomatic AD, but is also sensitive to AD-related biomarkers in healthy, asymptomatic individuals. Thus, we argue it is important to further explore the combination of attentional control and memory retrieval processes in cognitive tasks to gain a better understanding of the influence of AD-related pathology in cognitively normal older adults.

The primary limitation of this work is that it is cross-sectional in nature. Although the mediation analyses presented here are an attempt at understanding the causal mechanisms of CSF markers on cognitive outcomes, it must be emphasized that these correlational analyses do not indicate causality. We can, however, test whether patterns of correlations are consistent or inconsistent with a hypothesized model. Our results are consistent with a model in which the CSF biomarkers represent statistically independent processes that both influence cognition primarily through levels of amyloid measured with PIB. Differences in the reliability of each of the measures could also influence the pattern of correlations observed here.

Importantly, there is an emerging body of research that seeks to understand the interrelationships of the multiple biomarkers of AD. The present study extends this work by showing these same biomarkers influence cognition primarily through amyloid deposition. Furthermore, we show in a cognitively healthy control sample, the accumulating biomarkers impair performance in the same manner as very mild AD (CDR 0.5 participants). This further validates importance of developing sensitive behavioral and biological markers for the early detection of presymptomatic AD.

References

Aizenstein, H. J., Nebes, R. D., Saxton, J. A., Price, J. C., Mathis, C. A., Tsopelas, N. D., . . . Klunk, W. E. (2008). Frequent amyloid deposition without significant cognitive impairment among the elderly. *Archives of Neurology, 65,* 1509–1517. doi:10.1001/archneur.65.11.1509

Armitage, S. G. (1946). An analysis of certain psychological tests used in evaluation of brain injury. *Psychological Monographs: General and Applied, 60,* 1–48. doi:10.1037/h0093567

Balota, D. A., Burgess, G. C., Cortese, M. J., & Adams, D. R. (2002). The word-frequency mirror effect in young, old, and early-stage Alzheimer's disease: Evidence for two processes in episodic recognition. *Journal of Memory and Language, 46,* 199–226. doi:10.1006/jmla.2001.2803

Balota, D. A., & Duchek, J. M. (1991). Semantic priming effects, lexical repetition effects, and contextual disambiguation effects in healthy aged individuals and individuals with senile dementia of the Alzheimer type. *Brain and Language, 40,* 181–201. doi:10.1016/0093-934X(91)90124-J

Balota, D. A., & Faust, M. (2001). Attention in dementia of the Alzheimer's type. *Handbook of Neuropsychology, 6,* 51–80.

Balota, D. A., Tse, C. S., Hutchison, K. A., Spieler, D. H., Duchek, J. M., & Morris, J. C. (2010). Predicting conversion to dementia of the Alzheimer's type in a healthy control sample: The power of errors in Stroop color naming. *Psychology and Aging, 25,* 208–218. doi:10.1037/a0017474

Balota, D. A., Watson, J. M., Duchek, J. M., & Ferraro, F. R. (1999). Cross-modal semantic and homograph priming in healthy young, healthy old, and in Alzheimer's disease individuals. *Journal of the International Neuropsychological Society, 5,* 626–640. doi:10.1017/S1355617799577060

Berg, L., McKeel, D. W., Miller, J. P., Storandt, M., Rubin, E. H., Morris, J. C., . . . Saunders, A. M. (1998). Clinicopathologic studies in cognitively healthy aging and Alzheimer disease: Relation of histologic markers to dementia severity, age, sex, and Apolipoprotein E genotype. *Archives of Neurology, 55,* 326–335. doi:10.1001/archneur.55.3.326

Blacker, D., Haines, J. L., Rodes, L., Terwedow, H., Go, R. C. P., Harrell, L. E., . . . Tanzi, R. (1997). ApoE-4 and age at onset of Alzheimer's disease: The NIMH Genetics Initiative. *Neurology, 48,* 139–147. doi:10.1212/WNL.48.1.139

Canning, S. J. D., Leach, L., Stuss, D., Ngo, L., & Black, S. E. (2004). Diagnostic utility of abbreviated fluency measures in Alzheimer disease and vascular dementia. *Neurology, 62,* 556–562. doi:10.1212/WNL.62.4.556

Diniz, B. S. O., Pinto Jr., J. A., & Forlenza, O. V. (2008). Do CSF total tau, phosphorylated tau, and β-amyloid 42 help to predict progression of mild cognitive impairment to Alzheimer's disease? A systematic review and meta-analysis of the literature. *World Journal of Biological Psychiatry, 9,* 172–182. doi:10.1080/15622970701535502

Doraiswamy, P. M., Sperling, R. A., Coleman, R. E., Johnson, K. A., Reiman, E. M., Davis, M. D., . . . Pontecorvo, M. J. (2012). Amyloid-assessed by florbetapir F 18 PET and 18-month cognitive decline: A multicenter study. *Neurology, 79,* 1636–1644. doi:10.1212/WNL.0b013e3182661f74

Duchek, J. M., Balota, D. A., Thomas, J. B., Snyder, A. Z., Rich, P., Benzinger, T. L., . . . Ances, B. M. (2013). Relationship between Stroop performance and resting state functional connectivity in cognitively normal older adults. *Neuropsychology, 27,* 516–528. doi:10.1037/a0033402

Duchek, J. M., Balota, D. A., Tse, C. S., Holtzman, D. M., Fagan, A. M., & Goate, A. M. (2009). The utility of intraindividual variability in selective attention tasks as an early marker for Alzheimer's disease. *Neuropsychology, 23,* 746–758. doi:10.1037/a0016583

Fagan, A. M., Mintun, M. A., Shah, A. R., Aldea, P., Roe, C. M., Mach, R. H., . . . Holtzman, D. M. (2009). Cerebrospinal fluid tau and p-tau181 increase with cortical amyloid deposition in cognitively normal individuals: Implications for future clinical trials of Alzheimer's disease. *EMBO Molecular Medicine, 1,* 371–380. doi:10.1002/emmm.200900048

Fagan, A. M., Roe, C. M., Xiong, C., Mintun, M. A., Morris, J. C., & Holtzman, D. M. (2007). Cerebrospinal fluid tau/beta-amyloid42 ratio as a prediction of cognitive decline in nondemented older adults. *Archives of Neurology, 64,* 343–349. doi:10.1001/archneur.64.3.noc60123

Faust, M. E., & Balota, D. A. (2007). Inhibition, facilitation, and attentional control in dementia of the Alzheimer's type: The role of unifying principles in cognitive theory development. *Inhibition in Cognition,* 213–238.

Faust, M. E., Balota, D. A., Spieler, D. H., & Ferraro, F. R. (1999). Individual differences in information-processing rate and amount: Implications for group differences in response latency. *Psychological Bulletin, 125,* 777–799. doi:10.1037/0033-2909.125.6.777

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state": A practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research, 12,* 189–198. doi:10.1016/0022-3956(75)90026-6

Goodglass, H., & Kaplan, E. (1983). *Boston Diagnostic Aphasia Examination Booklet, III, ORAL EXPRESSION, J. Animal Naming (Fluency in Controlled Association).* Philadelphia: Lea & Febiger.

Grober, E., Buschke, H., Crystal, H., Bang, S., & Dresner, R. (1988). Screening for dementia by memory testing. *Neurology, 38,* 900–903. doi:10.1212/WNL.38.6.900

Hayes, A. F. (2013). *Introduction to mediation, moderation, and conditional process analysis: A regression-based approach.* New York, NY: Guilford Press.

Hedden, T., OH, H., Younger, A. P., & Patel, T. A. (2013). Meta-analysis of amyloid-cognition relations in cognitively normal older adults. *Neurology, 80,* 1341–1348. doi:10.1212/WNL.0b013e31828ab35d

Hodges, J. R., Salmon, D. P., & Butters, N. (1992). Semantic memory impairment in Alzheimer's disease: Failure of access or degraded knowledge? *Neuropsychologia, 30,* 301–314. doi:10.1016/0028-3932(92)90104-T

Hutchison, K. A., Balota, D. A., & Duchek, J. M. (2010). The utility of Stroop task switching as a marker for early-stage Alzheimer's disease. *Psychology and Aging, 25,* 545–559. doi:10.1037/a0018498

Jack, C. R., Lowe, V. J., Senjem, M. L., Weigand, S. D., Kemp, B. J., Shiung, M. M., . . . Peterson, R. C. (2008). 11C PiB and structural MRI provide complementary information in imaging of Alzheimer's disease and amnestic mild cognitive impairment. *Brain: A Journal of Neurology, 131,* 665–680. doi:10.1093/brain/awm336

Jacoby, L. L. (1999). Ironic effects of repetition: Measuring age-related differences in memory. *Journal of Experimental Psychology: Learning, Memory, and Cognition, 25,* 3–22. doi:10.1037/0278-7393.25.1.3

Jagust, W. J., Landau, S. M., Shaw, L. M., Trojanowski, J. Q., Koeppe, R. A., Reiman, E. M., . . . Mathis, C. A. (2009). Relationships between biomarkers in aging and dementia. *Neurology, 73,* 1193–1199. doi:10.1212/WNL.0b013e3181bc010c

Kirshner, H. S., Webb, W. G., & Kelly, M. P. (1984). The naming disorder of dementia. *Neuropsychologia, 22,* 23–30. doi:10.1016/0028-3932(84)90004-6

Klunk, W. E., Engler, H., Nordberg, A., Wang, Y., Blomqvist, G., Holt, D. P., . . . Langstrom, B. (2004). Imaging brain amyloid in Alzheimer's disease with Pittsburgh compound-B. *Annals of Neurology, 55,* 306–319. doi:10.1002/ana.20009

Logan, J., Fowler, J. S., Volkow, N. D., Wang, G.-J., Ding, Y-S., & Alexoff, D. L. (1996). Distribution volume ratios without blood sampling from graphical analysis of PET data. *Journal of Cerebral Blood Flow & Metabolism: Clinical and Experimental, 16,* 834–840. doi:10.1097/00004647-199609000-00008

MacDonald, A. W., Cohen, J. D., Stenger, V. A., & Carter, C. S. (2000). Dissociating the role of the dorsolateral prefrontal and anterior cingulate cortex in cognitive control. *Science, 288,* 1835–1838. doi:10.1126/science.288.5472.1835

MacLeod, C. M. (1991). Half a century of research on the Stroop effect: An integrative review. *Psychological Bulletin, 109,* 163–203. doi:10.1037/0033-2909.109.2.163

Marczinski, C. A., & Kertesz, A. (2006). Category and letter fluency in semantic dementia, primary progressive aphasia, and Alzheimer's disease. *Brain and Language, 97,* 258–265. doi:10.1016/j.bandl.2005.11.001

Mattsson, N., Andreasson, U., Persson, S., Arai, H., Batish, S. D., Bernardini, S., . . . Blennow, K. (2011). The Alzheimer's Association external quality control program for cerebrospinal fluid biomarkers. *Alzheimer's & Dementia, 7,* 386–395e6. doi:10.1016/j.jalz.2011.05.2243

Mintun, M. A., LaRossa, G. N., Sheline, Y. I., Dence, C. S., Lee, S. Y., Mach, R. H., . . . Morris, J. C. (2006). [11C]PIB in a nondemented population: Potential antecedent marker of Alzheimer disease. *Neurology, 67,* 446–452. doi:10.1212/01.wnl.0000228230.26044.a4

Mormino, E. C., Kluth, J. T., Madison, C. M., Rabinovici, G. D., Baker, S. L., Miller, B. L., . . . Jagust, W. J. (2009). Episodic memory loss is

related to hippocampal-mediated β-amyloid deposition in elderly subjects. *Brain: A Journal of Neurology, 132,* 1310–1323. doi:10.1093/brain/awn320

Morris, J. C. (1993). The Clinical Dementia Rating (CDR): Current version and scoring rules. *Neurology, 43,* 2412–2414. doi:10.1212/WNL.43.11.2412-a

Morris, J. C., Blennow, K., Froelich, L., Nordberg, A., Soininen, H., Waldemar, G., . . . Dubois, B. (2014). Harmonized diagnostic criteria for Alzheimer's disease: Recommendations. *Journal of Internal Medicine, 275,* 204–213. doi:10.1111/joim.12199

Morris, J. C., Roe, C. M., Xiong, C., Fagan, A. M., Goate, A. M., Holtzman, D. M., & Mintun, M. A. (2010). *APOE* predicts amyloid-beta but not tau Alzheimer pathology in cognitively normal aging. *Annals of Neurology, 67,* 122–131. doi:10.1002/ana.21843

Morris, J. C., Storandt, M., McKeel Jr., D. W., Rubin, E. H., Price, J. L., Grant, E. A., & Berg, L. (1996). Cerebral amyloid deposition and diffuse plaques in "normal" aging Evidence for presymptomatic and very mild Alzheimer's disease. *Neurology, 46,* 707–719. doi:10.1212/WNL.46.3.707

Mungas, D., Tractenberg, R., Schneider, J. A., Crane, P. K., & Bennett, D. A. (2014). A 2-process model for neuropathology of Alzheimer's disease. *Neurobiology of Aging, 35,* 301–308. doi:10.1016/j.neurobiolaging.2013.08.007

Nebes, R. D., Snitz, B. E., Cohen, A. D., Aizenstein, H. J., Saxton, J. A., Halligan, E. M., . . . Klunk, W. E. (2013). Cognitive aging in persons with minimal amyloid-β and white matter hyperintensities. *Neuropsychologia, 51,* 2202–2209. doi:10.1016/j.neuropsychologia.2013.07.017

Nelson, D. L., McEvoy, C. L., & Schreiber, T. A. (2004). The University of South Florida free association, rhyme, and word fragment norms. *Behavior Research Methods, Instruments & Computers, 36,* 402–407. doi:10.3758/BF03195588

Perry, R. J., & Hodges, J. R. (1999). Attention and executive deficits in Alzheimer's disease: A critical review. *Brain: A Journal of Neurology, 122,* 383–404. doi:10.1093/brain/122.3.383

Pike, K. E., Ellis, K. A., Villemagne, V. L., Good, N., Chételat, G., Ames, D., . . . Rowe, C. C. (2011). Cognition and beta-amyloid in preclinical Alzheimer's disease: Data from the AIBL study. *Neuropsychologia, 49,* 2384–2390. doi:10.1016/j.neuropsychologia.2011.04.012

Price, J. L., McKeel, Jr., D. W., Buckles, V. D., Roe, C. M., Xiong, C., Grundman, M., . . . Morris, J. C. (2009). Neuropathology of nondemented aging: Presumptive evidence for preclinical Alzheimer disease. *Neurobiology of Aging, 30,* 1026–1036. doi:10.1016/j.neurobiolaging.2009.04.002

Reiman, E. M., Chen, K., Liu, X., Bandy, D., Yu, M., Lee, W., . . . Caselli, R. J. (2009). Fibrillar amyloid-β burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. *Proceedings of the National Academy of Sciences, USA, 106,* 6820–6825.

Rodrigue, K. M., Kennedy, K. M., Devous, M. D., Rieck, J. R., Hebrank, A. C., Diaz-Arrastia, R., . . . Park, D. C. (2012). β-Amyloid burden in healthy aging regional distribution and cognitive consequences. *Neurology, 78,* 387–395. doi:10.1212/WNL.0b013e318245d295

Roe, C. M., Fagan, A. M., Grant, E. A., Hassenstab, J., Moulder, K. L., Dreyfus, D. M., . . . Morris, J. C. (2013). Amyloid imaging and CSF biomarkers in predicting cognitive impairment up to 7.5 years later. *Neurology, 80,* 1784–1791. doi:10.1212/WNL.0b013e3182918ca6

Rogers, T. T., Hocking, J., Noppeney, U., Mechelli, A., Gorno-Tempini, M. L., Patterson, K., & Price, C. J. (2006). Anterior temporal cortex and semantic memory: Reconciling findings from neuropsychology and functional imaging. *Cognitive, Affective & Behavioral Neuroscience, 6,* 201–213. doi:10.3758/CABN.6.3.201

Small, B. J., Rosnick, C. B., Fratiglioni, L., & Bäckman, L. (2004). Apolipoprotein E and cognitive performance: A meta-analysis. *Psychology and Aging, 19,* 592–600. doi:10.1037/0882-7974.19.4.592

Smith, E. E., Shoben, E. J., & Rips, L. J. (1974). Structure and process in semantic memory: A featural model for semantic decisions. *Psychological Review, 81,* 214–241. doi:10.1037/h0036351

Soldan, A., Pettigrew, C., Li, S., Wang, M-C., Moghekar, A., Selnes, O. A., . . . O'Brien, R. (2013). Relationship of cognitive reserve and cerebrospinal fluid biomarkers to the emergence of clinical symptoms in preclinical Alzheimer's disease. *Neurobiology of Aging, 34,* 2827–2834. doi:10.1016/j.neurobiolaging.2013.06.017

Sperling, R. A., Johnson, K. A., Doraiswamy, P. M., Reiman, E. M., Fleisher, A. S., Sabbagh, M. N., . . . Pontecorvo, M. J. (2013). Amyloid deposition detected with florbetapir F 18 (18F-AV-45) is related to lower episodic memory performance in clinically normal older individuals. *Neurobiology of Aging, 34,* 822–831. doi:10.1016/j.neurobiolaging.2012.06.014

Spieler, D. H., Balota, D. A., & Faust, M. E. (1996). Stroop performance in healthy younger and older adults and in individuals with dementia of the Alzheimer's type. *Journal of Experimental Psychology: Human Perception and Performance, 22,* 461–479. doi:10.1037/0096-1523.22.2.461

Stern, Y. (2009). Cognitive reserve. *Neuropsychologia, 47,* 2015–2028. doi:10.1016/j.neuropsychologia.2009.03.004

Storandt, M., Balota, D. A., Aschenbrenner, A. J., & Morris, J. C. (2014). Clinical and psychological characteristics of the initial cohort of the Dominantly Inherited Alzheimer Network (DIAN). *Neuropsychology, 28,* 19–29. doi:10.1037/neu0000030

Storandt, M., Grant, E. A., Miller, J. P., & Morris, J. C. (2006). Longitudinal course and neuropathologic outcomes in original vs. revised MCI and in pre-MCI. *Neurology, 67,* 467–473.

Storandt, M., Head, D., Fagan, A. M., Holtzman, D. M., & Morris, J. C. (2012). Toward a multifactorial model of Alzheimer disease. *Neurobiology of Aging, 33,* 2262–2271. doi:10.1016/j.neurobiolaging.2011.11.029

Storandt, M., Mintun, M. A., Head, D., & Morris, J. C. (2009). Cognitive decline and brain volume loss as signatures of cerebral amyloid-β peptide deposition identified with Pittsburgh compound B. *Archives of Neurology, 66,* 1476–1481. doi:10.1001/archneurol.2009.272

Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *Journal of Experimental Psychology, 18,* 643–662. doi:10.1037/h0054651

Su, Y., D'Angelo, G. M., Vlassenko, A. G., Zhou, G., Snyder, A. Z., Marcus, D. S., . . . Benzinger, T. L. S. (2013). Quantitative analysis of PiB-PET with FreeSurfer ROIs. *PLoS ONE, 8*(11), e73377. doi:10.1371/journal.pone.0073377

Tse, C. S., Balota, D. A., Moynan, S. C., Duchek, J. M., & Jacoby, L. L. (2010). The utility of placing recollection in opposition to familiarity in early discrimination of healthy aging and very mild dementia of the Alzheimer's type. *Neuropsychology, 24,* 49–67. doi:10.1037/a0014887

Tse, C. S., Balota, D. A., Yap, M. J., Duchek, J. M., & McCabe, D. P. (2010). Effects of healthy aging and early stage dementia of the Alzheimer's type on components of response time distributions. *Neuropsychology, 24,* 300–315. doi:10.1037/a0018274

Twamley, E. W., Ropacki, S. A. L., & Bondi, M. W. (2006). Neuropsychological and neuroimaging changes in preclinical Alzheimer's disease. *Journal of the International Neuropsychological Society, 12,* 707–735. doi:10.1017/S1355617706060863

Van Overschelde, J. P., Rawson, K. A., & Dunlosky, J. (2004). Category norms: An updated and expanded version of the Battig and Montague (1969) norms. *Journal of Memory and Language, 50,* 289–335. doi:10.1016/j.jml.2003.10.003

Vemuri, P., Weigand, S. D., Przybelski, S. A., Knopman, D. S., Smith, G. E., Trojanowski, J. Q., . . . Jack, C. R. (2011). Cognitive reserve and Alzheimer's disease biomarkers are independent determinants of cognition. *Brain: A Journal of Neurology, 134,* 1479–1492. doi:10.1093/brain/awr049

Vemuri, P., Wiste, H. J., Weigand, S. D., Shaw, L. M., Trojanowski, J. Q., Weiner, M. W., . . . Jack, C. R. (2009). MRI and CSF biomarkers in normal, MCI, and AD subjects: Diagnostic discrimination and cognitive correlations. *Neurology, 73,* 287–293. doi:10.1212/WNL.0b013e3181af79e5

Wechsler, D. (1997). *WAIS-III administration and scoring manual*. San Antionio, TX: Psychological Corporation.

Wisdom, N. M., Callahan, J. L., & Hawkins, K. A. (2011). The effects of apolipoprotein E on non-impaired cognitive functioning: A meta-analysis. *Neurobiology of Aging, 32,* 63–74. doi:10.1016/j.neurobiolaging.2009.02.003

Received March 24, 2014
Revision received June 13, 2014
Accepted July 7, 2014 ■