



Stroke risk interacts with Alzheimer's disease biomarkers on brain aging outcomes



Timothy J. Hohman^{a,*}, Lauren R. Samuels^{a,b}, Dandan Liu^{a,b}, Katherine A. Gifford^a, Shubhabrata Mukherjee^c, Elleena M. Benson^a, Ty Abel^d, Frederick L. Ruberg^e, Angela L. Jefferson^a, for the Alzheimer's Neuroimaging Initiative¹

^a Vanderbilt Memory and Alzheimer's Center, Vanderbilt University School of Medicine, Nashville, TN, USA

^b Department of Biostatistics, Vanderbilt University School of Medicine, Nashville, TN, USA

^c Department of Medicine, University of Washington, Seattle, WA, USA

^d Department of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, TN, USA

^e Section of Cardiovascular Medicine, Department of Medicine, Boston University School of Medicine and Boston Medical Center, Boston, MA, USA

ARTICLE INFO

Article history:

Received 19 March 2015

Received in revised form 28 May 2015

Accepted 30 May 2015

Available online 6 June 2015

Keywords:

Stroke risk
Alzheimer's disease
Biomarkers
Cognition
Hippocampus
Cerebrovascular disease
Aging

ABSTRACT

Alzheimer's disease (AD) biomarkers and stroke risk factors independently predict cognitive impairment, likely through independent disease pathways. However, limited work has sought to describe the dynamic interplay between these important risk factors. This article evaluated the interaction between stroke risk and AD biomarkers on hippocampal volume and cognitive performance. We first evaluated the interaction between stroke risk factors and AD biomarkers using data from the Alzheimer's Disease Neuroimaging Initiative (ADNI, $n = 1202$). We then extended our findings to an independent autopsy data set from the National Alzheimer's Coordinating Center (NACC, $n = 1122$) using measures of AD pathology. Stroke risk was quantified using the Framingham Stroke Risk Profile. In ADNI, stroke risk interacted with tau and amyloid levels in relation to baseline and longitudinal cognitive performance. Similarly, in NACC, stroke risk interacted with amyloid and tau positivity on cognitive performance. The effect of stroke risk factors on cognition was strongest in the absence of AD biomarkers or neuropathology, providing additional evidence that AD biomarkers and stroke risk factors relate to cognition through independent pathways.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Stroke risk factors, such as hypertension and cigarette smoking, have been associated with lower neuropsychological performance in elders with normal cognition (NC) and mild cognitive impairment (MCI; Brady et al., 2001) and in relation to incident Alzheimer's disease (AD; Kivipelto et al., 2002). Although much AD work has focused on classifying “pure” AD in the absence of vascular disease (Jack et al., 2010, 2013), the autopsy literature has

clearly demonstrated that the most common presentation of AD is a mixed pathology with contributions from amyloid- β ($A\beta$) plaques, tau neurofibrillary tangles, and cerebrovascular disease (Schneider et al., 2007a; Schneider and Bennett, 2010; Troncoso et al., 2008). The dynamic interplay among AD and cerebrovascular pathologies remains somewhat elusive, but their co-occurrence leaves open the possibility that risk factors for both may interact in conferring risk for neurodegeneration (e.g., hippocampal volume) and cognitive decline (e.g., neuropsychological performance).

Cerebrospinal fluid (CSF) biomarkers of AD include $A\beta$ -42, total tau, and phosphorylated tau levels based on their strong associations with brain volume (de Souza et al., 2012; Fjell et al., 2010) neuropsychological impairment (Burger et al., 2005; Jagust et al., 2009), and postmortem AD pathology (Burger et al., 2006). Similarly, stroke risk factors have shown strong associations with brain volume (Seshadri et al., 2004), neuropsychological impairment (Brady et al., 2001; Jefferson et al., 2015; Kivipelto et al., 2002), and cerebrovascular pathology (Wang et al., 2009; Wolf et al., 1991). In mouse models, there has been some evidence that certain stroke

* Corresponding author at: Vanderbilt Memory and Alzheimer's Center, Vanderbilt University School of Medicine, 2525 West End Ave, 12th Floor, Suite 1200, Nashville, TN 37203, USA. Tel.: 615 343 8429; fax: 615-322-6106.

E-mail address: Timothy.J.Hohman@Vanderbilt.edu (T.J. Hohman).

¹ Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

risk factors, such as smoking (Moreno-Gonzalez et al., 2013) and hypertension (Diaz-Ruiz et al., 2009; Gentile et al., 2009), actually exacerbate AD pathology. Thus, there may be differing effects of stroke risk factors on neurodegeneration depending on the presence or absence of AD biomarkers. Yet, despite the depth of research investigating AD biomarkers and stroke risk factors independently, less research has focused on evaluating whether these factors interact in relation to brain volume or neuropsychological performance.

The present study examines the interplay between stroke risk factors and CSF biomarkers in relation to cross-sectional and longitudinal measures of brain aging. First, in the Alzheimer's Disease Neuroimaging Initiative (ADNI) data set, we evaluate interactions between stroke risk factors and AD biomarkers in relation to cross-sectional and longitudinal hippocampal volume. Second, we test the same interactions in relation to cross-sectional and longitudinal neuropsychological performance. Finally, we replicate the observed interaction effects on neuropsychological performance in a second, independent cohort using the National Alzheimer's Coordinating Center (NACC) data set. We could not analyze hippocampal volume in NACC because magnetic resonance imaging data were not available for analysis, so replication analyses focus on cognitive performance. Our hypothesis was that the effect of stroke risk factors on brain aging outcomes would depend on AD biomarker levels whereby vascular risk would exacerbate brain aging in the presence of AD biomarkers.

2. Materials and methods

Data used in the preparation of this article were obtained from the ADNI launched in 2003 (adni.loni.usc.edu). The initial goal of ADNI was to recruit 800 subjects but ADNI has been followed by ADNI-GO and ADNI-2. To date these 3 protocols have recruited >1500 adults, ages 55–90 years, excluding serious neurological disease other than AD, history of brain lesion or head trauma, and history of psychoactive medication use (for full inclusion and/or exclusion criteria see www.adni-info.org). Informed written consent was obtained from all participants at each site.

The replication sample was drawn from the NACC, which maintains a database of participant information collected from 34 past and present National Institute on Aging-funded Alzheimer's Disease Centers. In 2005, NACC implemented a standard protocol (i.e., Uniform Data Set), including clinical, medical, neurological, and neuropsychological data (Beekly et al., 2004). Analysis of both publically available databases was approved by our local Institutional Review Board.

2.1. Participants

We accessed publicly available data from ADNI on June 1, 2014. Participants were enrolled based on criteria outlined in the ADNI protocol (<http://www.adni-info.org/Scientists/AboutADNI.aspx>). For the present analyses, we included all participants who had CSF biomarker data, full vascular risk factor data needed to calculate a stroke risk score, and the outcome measure of interest. For the neuroimaging analyses, participants had to have a FreeSurfer measure of hippocampal volume derived from 1.5 T magnetic resonance imaging data, yielding 1082 participants. For cognitive analyses, participants had to have a composite measure of episodic memory and EF, yielding 1202 participants with all measures of interest.

For the replication sample, we used neuropathology data because NACC does not have CSF biomarker data available for analysis. NACC participants between 55 and 90 years of age evaluated between September 1, 2005 and September 29, 2014 with neuropathology,

neuropsychological, and stroke risk data were included, yielding 1122 participants.

2.2. Framingham Stroke Risk Profile

To assess systemic vascular health, we calculated Framingham Stroke Risk Profile (FSRP) at baseline in the ADNI data set and at the last visit before death in the NACC data set. FSRP assigns points for age, systolic blood pressure (accounting for antihypertensive treatment), history of diabetes, current cigarette smoking, prevalent cardiovascular disease (i.e., history of myocardial infarction, angina pectoris, coronary insufficiency, intermittent claudication, or heart failure), left ventricular hypertrophy, and history of atrial fibrillation (D'Agostino et al., 1994). In this study, the FSRP calculation is modified because left ventricular hypertrophy information is not available in ADNI or in NACC. FSRP values range from 0 to 44 with higher values indicating more adverse risk of stroke.

2.3. CSF biomarker processing (ADNI) and autopsy measures of neuropathology (NACC)

ADNI's CSF protocol, including the quantification of A β -42 and tau, has been outlined in detail elsewhere (Jagust et al., 2009; Shaw et al., 2011). For the present analyses, we compiled a data set across the UPENN1–UPENN5 data sources available for download on the ADNI site and used the first measure of total tau and A β -42 available for each subject. Biomarker levels were entered as continuous predictors in statistical models.

In the NACC data set, we used the semiquantitative Consortium to Establish a Registry for Alzheimer's Disease neuritic plaque staging to classify participants as either "amyloid positive" or "amyloid negative". Individuals with no plaques or sparse plaques were considered amyloid negative, and those with moderate or frequent plaques were considered amyloid positive. Similarly, we used the semiquantitative Braak neurofibrillary tangle staging to identify participants as either "tau positive" or "tau negative." Braak stages 0, I, and II were considered "tau negative," and Braak stages III–VI were considered "tau positive."

2.4. Composite neuropsychological measurements

The ADNI neuropsychological protocol has been reported in detail, including calculation of composite measures of episodic memory and EF (Crane et al., 2012; Gibbons et al., 2012). We leveraged both the memory (ADNI-MEM) and the executive function (ADNI-EF) scores in the present analyses. Briefly, a single factor model based on item level data from the Rey Auditory Verbal Learning Test, the AD Assessment Scale–Cognitive Subscale, the Mini-Mental State Examination, and the Logical Memory test were used in the calculation of the ADNI-MEM score. Item level data from the Trail Making Test (A and B), digit span backward, digit symbol, animal naming, vegetable naming, and the clock drawing test were used in the calculation of the ADNI-EF score.

We used a well-validated psychometric approach to calibrate memory and executive functioning scores from the ADNI and NACC databases (Crane et al., 2008). Cocalibration refers to combining test scores across studies into a single metric. Briefly, we cocalibrated ADNI-MEM and ADNI-EF scores with NACC item level data to obtain NACC memory (NACC-MEM) and executive function (NACC-EF) scores on the same metric as ADNI using previously published methods (Crane et al., 2008; Mez et al., Under Review). Common memory measures in NACC and ADNI (i.e., Logical Memory Immediate and Delayed Recall) and common EF measures (i.e., digit span backwards, verbal fluency, vegetable fluency, and Trail Making Test parts A and B) served as anchors for cocalibration. We calculated

Table 1
Sample characteristics

	Baseline clinical diagnosis ^a			F-test
	Normal control	Mild cognitive impairment	Alzheimer's disease	
ADNI: brain volume data set				
Sample size, n	342	558	182	n/a
APOE ε4 carriers, %	27	49	68	n/a
Females, %	53	41	46	n/a
Baseline age, y	74 ± 6	72 ± 7	74 ± 8	F(2,1079) = 7.31, <i>p</i> < 0.001
Education, y	16 ± 3	16 ± 3	15 ± 3	F(2,1079) = 6.33, <i>p</i> = 0.002
Visits, total	3 ± 2	4 ± 2	2 ± 1	F(2,1079) = 38.64, <i>p</i> < 0.001
Stroke risk profile score	13 ± 4	12 ± 4	13 ± 5	F(2,1079) = 4.79, <i>p</i> = 0.009
CSF total tau, pg/mL	67 ± 30	91 ± 56	131 ± 62	F(2,1079) = 94.59, <i>p</i> < 0.001
CSF P-tau, pg/mL	32 ± 19	39 ± 22	52 ± 29	F(2,1074) = 47.14, <i>p</i> < 0.001
CSF Aβ-42, pg/mL	200 ± 52	172 ± 53	140 ± 41	F(2,1079) = 84.82, <i>p</i> < 0.001
Left hippocampal volume	3698 ± 436	3360 ± 604	2883 ± 540	F(2,1079) = 133.99, <i>p</i> < 0.001
ADNI: cognition data set				
Sample size, n	369	607	226	n/a
APOE ε4 carriers, %	27	49	67	n/a
Females, %	53	41	42	n/a
Baseline age, y	74 ± 6	73 ± 8	75 ± 8	F(2,1199) = 8.98, <i>p</i> < 0.001
Education, y	16 ± 3	16 ± 3	15 ± 3	F(2,1199) = 8.08, <i>p</i> < 0.001
Visits, total	7 ± 8	7 ± 7	3 ± 1	F(2,1199) = 32.41, <i>p</i> < 0.001
Stroke risk profile score	13 ± 4	12 ± 4	13 ± 4	F(2,1199) = 5.11, <i>p</i> = 0.006
CSF total tau, pg/mL	68 ± 32	91 ± 56	127 ± 62	F(2,1199) = 96.24, <i>p</i> < 0.001
CSF P-tau, pg/mL	32 ± 19	39 ± 22	51 ± 30	F(2,1194) = 48.46, <i>p</i> < 0.001
CSF Aβ-42, pg/mL	200 ± 52	172 ± 53	140 ± 39	F(2,1199) = 99.38, <i>p</i> < 0.001
Episodic memory	0.94 ± 0.51	0.19 ± 0.592	-0.71 ± 0.513	F(2,1199) = 626.92, <i>p</i> < 0.001
Executive function	0.78 ± 0.72	0.21 ± 0.798	-0.83 ± 0.82	F(2,1199) = 297, <i>p</i> < 0.001
NACC: cognition replication data set				
Sample size, n	240	110	772	n/a
APOE ε4 carriers, %	14	24	44	n/a
Females, %	62	46	45	n/a
Age at death, y	86 ± 9	83 ± 9	83 ± 9	F(2,1119) = 9.01, <i>p</i> < 0.001
Education, y	15 ± 3	15 ± 3	15 ± 3	F(2,1119) = 2.17, <i>p</i> = 0.114
Visits, total	2.84 ± 1.55	2.07 ± 1.276	2.66 ± 1.493	F(2,1119) = 10.19, <i>p</i> < 0.001
Time to death, y	1.47 ± 1.13	2.06 ± 1.544	1.82 ± 1.377	F(2,1119) = 8.92, <i>p</i> < 0.001
Stroke risk profile score	16 ± 4	15 ± 5	15 ± 4	F(2,1119) = 11.03, <i>p</i> < 0.001
Episodic memory	0.17 ± 0.165	0 ± 0.197	-0.26 ± 0.177	F(2,1119) = 579.53, <i>p</i> < 0.001
Executive function	0.08 ± 0.736	-0.74 ± 0.8	-1.52 ± 0.903	F(2,1119) = 325.26, <i>p</i> < 0.001

Key: ADNI, Alzheimer's Disease Neuroimaging Initiative; APOE ε4, apolipoprotein E epsilon 4; CSF, Cerebrospinal fluid; NACC, National Alzheimer's Coordinating Center.

^a Diagnostic groups were defined according to the ADNI protocol. Normal control participants had a Mini-Mental State Examination (MMSE) score between 24 and 30, a Clinical Dementia Rating (CDR) score of 0, and were not depressed (Geriatric Depression Scale score < 6). Mild cognitive impairment participants had an MMSE score between 24 and 30, objective memory impairment, subjective memory impairment, and a CDR score of 0.5. Alzheimer's disease participants met clinical criteria for dementia, had an MMSE of between 20 and 26, and had CDR score of 0.5 or 1.

the memory and EF scores for all NACC participants using parameters from ADNI-Mem and ADNI-EF. We performed cocalibration using Mplus software (Muthén and Muthén, 1998).

2.5. Quantification of hippocampal volume and hippocampal atrophy

The ADNI neuroimaging protocol has been reported in detail elsewhere (Jack et al., 2008). Images for the present study included original uncorrected 1.5 T T1-weighted high-resolution 3-dimensional structural data. Cortical reconstruction and volumetric segmentation were performed with the FreeSurfer image analysis suite version 4.3 in ADNI-1 and 5.1 in ADNI-2 (<http://surfer.nmr.mgh.harvard.edu/>) (Dale et al., 1999; Fischl et al., 1999a, 1999b). FreeSurfer processing in ADNI has been described in detail elsewhere (Mormino et al., 2009). An early version of the longitudinal image processing framework was used to process the sequential scans (Reuter et al., 2012). We used left hippocampal volume as our primary outcome measurement and included a measurement of intracranial volume as a covariate in all volumetric analyses, both of which were defined in FreeSurfer (Desikan et al., 2006). Mixed model regression, described in the following section, was used to model annual change in brain volume. Neuroimaging data are not available in the NACC data set.

2.6. Statistical analyses

All statistical analyses were performed in R (version 2.15.2; <http://www.r-project.org/>). Our threshold for statistical significance was set a priori at $\alpha < 0.05$. In all analyses, covariates consisted of age, sex, education, diagnostic status (NC, MCI, and AD), and intracranial volume (when applicable). Cross-sectional analyses were performed using baseline data within a general linear model (R command glm). Longitudinal analyses were performed using a mixed-effects regression model (R package nlme, R command lme) with time modeled as years from baseline for each participant (days from baseline/365.25). Main effects of each CSF biomarker and the FSRP were performed first using separate models to establish associations between our variables of interest and selected phenotypes. Demographic characteristics were compared across diagnoses using 1-way analysis of variance, and post hoc paired comparisons were performed using an independent samples *t* test correcting for multiple comparisons.

2.6.1. FSRP × CSF biomarker levels on hippocampal volume

The baseline hippocampal volume model included a term for the CSF biomarker of interest (1 model for Aβ-42, 1 model for total tau [t-tau], and 1 model for phosphorylated tau [p-tau]), a term for FSRP score, and a biomarker × FSRP interaction term. The

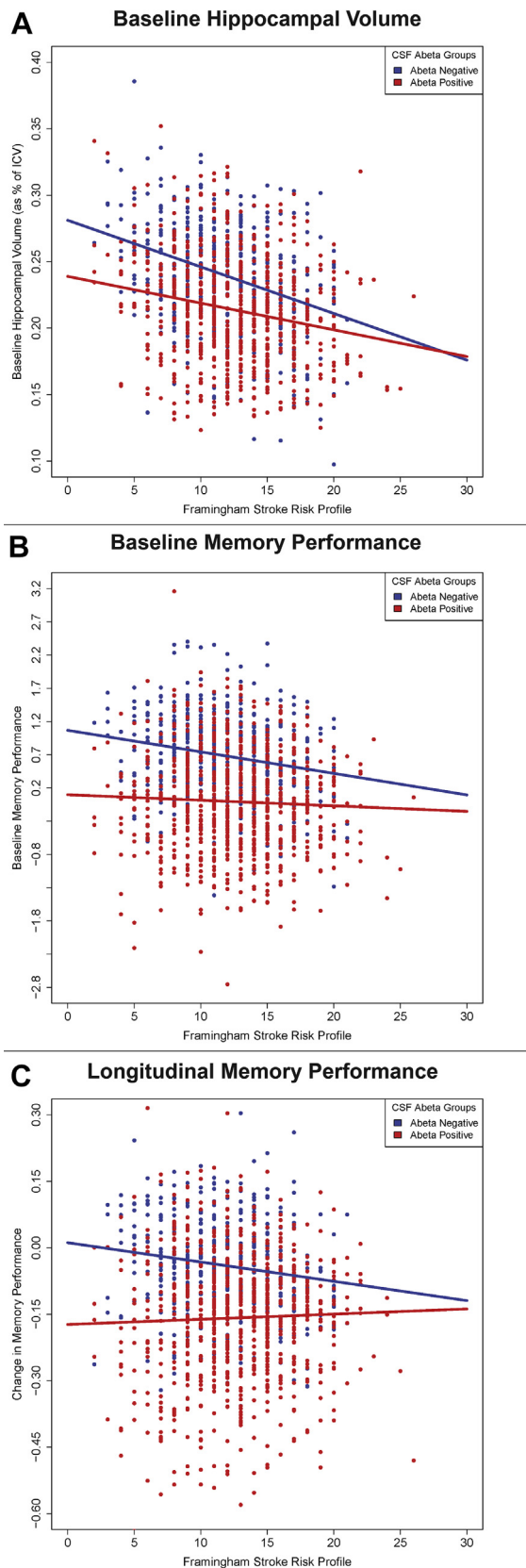


Fig. 1. CSF amyloid beta ($A\beta$)-42 interacts with stroke risk on brain aging. Framingham Stroke Risk Profile is along the x-axis and $A\beta$ -42 groups are split based on a previously identified cut point ($A\beta$ -42 positive ≤ 192). In row A, baseline left hippocampal volume is along the y-axis. In row B, baseline memory performance is along the y-axis. In row C, annual change in memory performance is along the y-axis. Abbreviations: CSF, cerebrospinal fluid; ICV, intracranial volume.

longitudinal hippocampal volume model included identical terms but also included a 3-way biomarker \times FSRP \times time interaction term (with all lower order terms included) to evaluate the interaction effects on change in hippocampal volume over the follow-up period.

Post hoc analyses evaluating 3-way diagnosis \times FSRP \times CSF biomarker interactions were run at baseline and longitudinally to assess whether the observed interaction effects differed between diagnostic categories. Additional subanalyses were run evaluating interactions in relation to each neuropsychological test that went into our composite measures of cognition, and evaluating both $A\beta$ -42 and t-tau in the same statistical model.

2.6.2. FSRP \times CSF biomarker levels on cognitive performance

The baseline and longitudinal cognitive models included the same interaction and adjusting covariate terms listed previously. One model was run with memory performance (ADNI-MEM) set as the outcome, and 1 model was run with EF performance (ADNI-EF) set as the outcome. The same post hoc tests previously mentioned were run.

2.6.3. NACC replication: FSRP \times autopsy measures of AD pathology on cognitive performance

In NACC, because we did not have baseline biomarker data, we evaluated the FSRP \times neuropathology interactions in 2 ways to best approximate the models tested in ADNI. First, we used a cross-sectional model leveraging cognitive data from the final visit before death to replicate the cross-sectional study in the initial analyses. Second, we used a longitudinal model leveraging all cognitive data points to evaluate trajectories of cognitive performance before death. In the longitudinal model, time was modeled as years from death. The model also included age at final visit, sex, diagnosis at final visit, and education. Composite measures of cognition (NACC-EF and NACC-MEM) were set as quantitative outcomes in each statistical model.

3. Results

Demographic data are presented in Table 1. In ADNI, AD participants differed as expected compared with participants with NC including a lower percentage of females, lower education levels, enhanced AD biomarker levels, lower cognitive performance levels, and smaller hippocampal volumes. In ADNI, stroke risk score was lowest in MCI participants but did not differ between AD and NC. In NACC, the AD participants had a lower percentage of females and showed lower cognitive performance levels than NC and MCI participants, but AD and MCI participants tended to be younger than NC participants. NC participants also tended to have slightly higher FSRP than MCI or AD.

3.1. Main effects of FSRP and AD biomarkers

At baseline, CSF $A\beta$ -42 [$t(1115) = 5.65, p < 0.001$] and CSF t-tau [$t(1080) = -4.38, p < 0.001$] were associated with hippocampal volume, whereas FSRP ($p = 0.084$) and p-tau were not associated ($p = 0.278$). CSF $A\beta$ -42 [$t(1236) = 8.48, p < 0.001$], CSF t-tau [$t(1201) = -3.82, p < 0.001$], CSF p-tau [$t(1232) = -4.92, p < 0.001$], and FSRP [$t(1716) = -2.21, p = 0.03$] were associated with baseline memory performance. Similarly, CSF $A\beta$ -42 [$t(1236) = 7.15, p < 0.001$], CSF t-tau [$t(1201) = -6.53, p < 0.001$], CSF p-tau [$t(1232) = -2.74, p = 0.006$], and FSRP [$t(1716) = -4.43, p < 0.001$] were all associated with baseline EF performance.

When evaluating longitudinal change, CSF $A\beta$ -42 [$t(2614) = 9.34, p < 0.001$], CSF t-tau [$t(2544) = -8.28, p < 0.001$], and CSF p-tau [$t(2609) = -8.44, p < 0.001$] were associated with

Table 2
Associations between FSRP and brain aging variables

	FSRP		FSRP × Aβ-42		FSRP × tau		FSRP × P-tau		
	β	p-value	β	p-value	β	p-value	β	p-value	
Baseline outcomes									
Hippocampal volume	-7.02	0.084	-0.19	0.005	-0.02	0.753	0.04	0.775	
Episodic memory composite	-0.01	0.027	-0.0002	0.0004	0.0001	0.035	0.0004	0.006	
Executive function composite	-0.028	3.19 × 10⁻⁶	-0.0002	0.085	0.00008	0.402	0.0003	0.256	
Longitudinal outcomes									
Hippocampal volume	-0.132	0.823	-0.005	0.719	0.026	0.060	-0.005	0.885	
Episodic memory composite	-0.003	0.021	-0.00005	0.098	0.00007	0.017	0.0002	0.038	
Executive function composite	0.0002	0.896	-0.00009	0.020	0.00009	0.030	0.0002	0.072	
NACC replication: autopsy sample									
	FSRP		FSRP × amyloid positivity		FSRP × tau positivity				
	β	p-value	β	p-value	β	p-value			
Last visit before death outcomes									
Episodic memory composite	-0.0008	0.538	0.005	0.027	-0.00002	0.991			
Executive function composite	0.0003	0.970	0.022	0.048	0.025	0.024			
Longitudinal outcomes									
Episodic memory composite	-0.0007	0.083	-0.00003	0.972	0.001	0.115			
Executive function composite	-0.003	0.112	0.005	0.202	-0.003	0.362			

Bold values signify effects that are significant at $p < 0.05$.

Key: FSRP, Framingham Stroke Risk Profile.

hippocampal atrophy, whereas FSRP was not ($p = 0.83$). All variables of interest were associated with faster memory decline, including CSF Aβ-42 [$t(6535) = 11.43, p < 0.001$], CSF t-tau [$t(1201) = -10.57, p < 0.001$], CSF p-tau [$t(6508) = -6.64, p < 0.001$], and FSRP [$t(10,628) = -2.31, p = 0.021$]. Additionally, Aβ-42 [$t(6499) = 11.60, p < 0.001$], t-tau [$t(6416) = -9.79, p < 0.001$], and p-tau [$t(6472) = -7.30, p < 0.001$] were associated with faster decline in EF, but FSRP showed no association ($p = 0.90$).

3.2. Interaction between FSRP and AD biomarkers on hippocampal volume

In baseline analyses, there was a significant interaction between FSRP and Aβ-42 [$t(1108) = -2.81, p = 0.005$] on hippocampal volume in which the relation between stroke risk and smaller hippocampal volume was strongest in individuals with lower brain amyloid burden (higher CSF Aβ-42 levels; Fig. 1). It should be noted that, lower CSF Aβ-42 is indicative of higher brain amyloid burden (Jagust et al., 2009). In longitudinal analyses, there were no significant interactions. Results are presented in Table 2.

3.3. Interaction between FSRP and AD biomarkers on cognition

In baseline analyses, there was a significant interaction effect between FSRP and Aβ-42 [$t(1229) = -2.95, p = 0.003$] on memory performance, whereby the relation between stroke risk and worse cognitive performance was strongest in the presence of lower brain amyloid burden (higher CSF Aβ-42 levels; Fig. 1). However, the worst performance was observed in those with both biomarker positivity and high stroke risk (Fig. 2). There was also a significant FSRP × Aβ-42 × diagnosis interaction [$t(1223) = -2.06, p = 0.024$] by which the interaction between stroke risk and Aβ-42 was strongest in MCI participants. There were no significant interactions in relation to baseline EF.

In longitudinal analyses, there was a significant interaction between FSRP × t-tau × interval [$t(6436) = 2.38, p = 0.017$] and FSRP × p-tau × interval [$t(6493) = 2.08, p = 0.038$] on longitudinal memory performance. In both cases, the relation between stroke risk and worsening cognition was strongest in biomarker negative individuals (those with lower CSF t-tau and p-tau). There was also a significant interaction between FSRP × Aβ-42 × interval [$t(6484) = -2.32, p = 0.02$] on EF performance and between FSRP ×

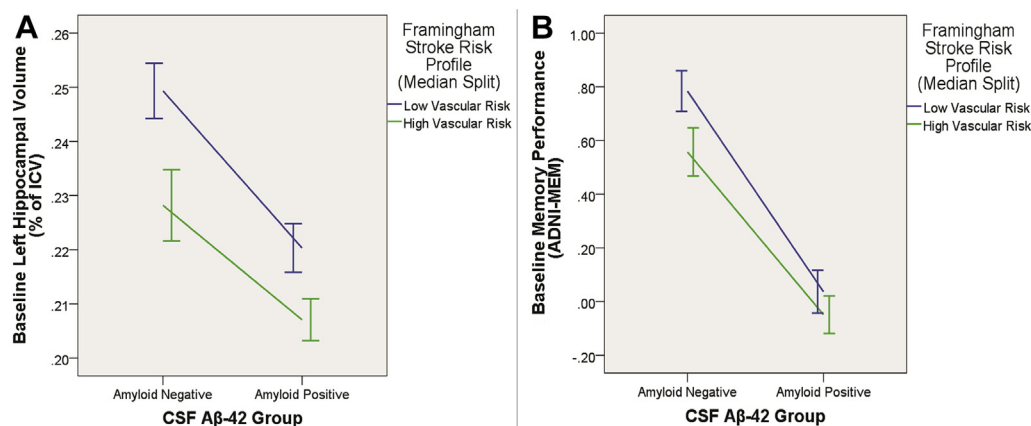


Fig. 2. The presence of high stroke risk and Alzheimer' disease biomarker positivity is related to low hippocampal volumes and memory performance scores at baseline. CSF Aβ-42 group is along the x-axis and groups are separated based on a median split of the Framingham Stroke Risk Profile score. Error bars represent the 95% confidence intervals. In panel A, baseline hippocampal volume is along the y-axis. In panel B, baseline memory performance is along the y-axis. Abbreviations: Aβ-42, amyloid beta 42; ADNI, Alzheimer's Disease Neuroimaging Initiative; CSF, cerebrospinal fluid; ICV, intracranial volume.

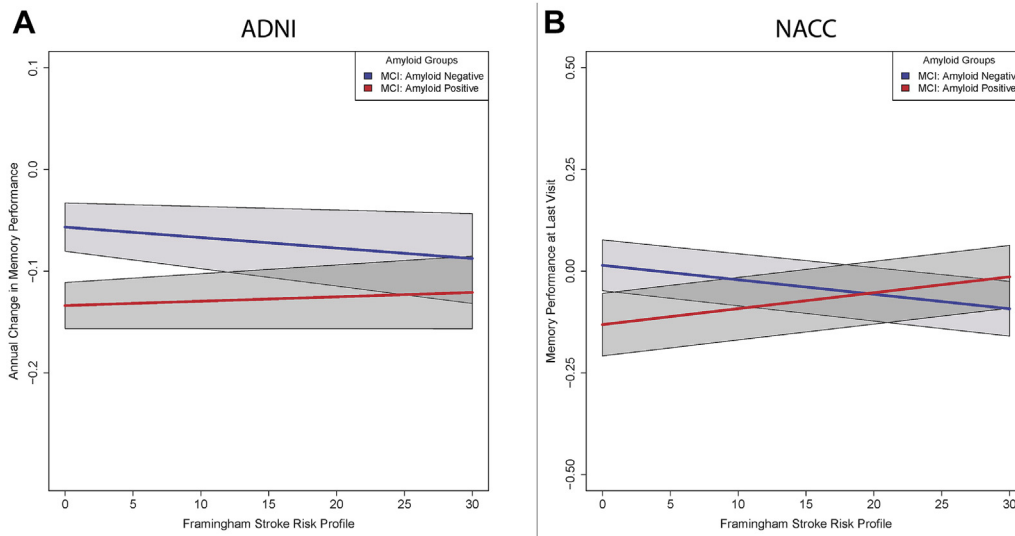


Fig. 3. Fitted plots demonstrating interaction between Framingham Stroke Risk Profile (FSRP) and amyloid on memory performance in ADNI and NACC. Graphs illustrate the predicted trajectories from the full regression model correcting for age, sex, education, and diagnosis. Fitted models are for a male participant of average age and average education level with MCI. FSRP is along the x-axis. In panel A, annual change in composite memory performance is along the y-axis and groups are defined based on cerebrospinal fluid (CSF) amyloid beta ($A\beta$ -42 levels ($A\beta$ -42 positive ≤ 192). In panel B, composite memory performance at last visit is along the y-axis and groups are defined based on CERAD neuritic plaque score in which no or sparse plaques are considered amyloid negative and moderate or frequent plaques are considered amyloid positive. Shaded regions represent the 95% confidence interval for the regression line. Abbreviations: ADNI, Alzheimer's Disease Neuroimaging Initiative; MCI, mild cognitive impairment; NACC, National Alzheimer's Coordinating Center.

t-tau \times interval [$t(6401) = 2.18, p = 0.03$] on EF performance. There were no significant diagnostic interactions. Results are presented in Table 2. Secondary subanalyses across each of the neuropsychological tests that went into the composite measures of cognition are presented in Supplementary Table 1. Secondary analyses evaluating $A\beta$ -42 and t-tau in the same statistical model are presented in Supplementary Table 2.

3.4. NACC replication of FSRP \times biomarker interaction on neuropsychological performance using autopsy measures of pathology

Replication results are presented in Table 2. FSRP was not cross-sectionally or longitudinally related to memory or EF performance in NACC. However, we did observe a significant FSRP \times diagnosis interaction in relation to EF performance before death [$F(2,1113) = 13.56, p < 0.001$] with a strong main effect of FSRP in individuals with NC, a weaker effect in MCI, and no effect in individuals with AD.

In cross-sectional replication analyses leveraging cognitive data from the last visit before death, FSRP interacted with amyloid positivity on memory performance [$t(1113) = 2.21, p = 0.027$] and EF [$t(1113) = 1.98, p = 0.048$], and interacted with tau positivity on EF [$t(1113) = 2.27, p = 0.024$]. The FSRP effect was again strongest in tau negative participants and amyloid negative participants, replicating the ADNI finding (Fig. 3). There were no 3-way interactions among pathology, diagnosis, and FSRP.

In longitudinal replication analyses, FSRP did not interact with amyloid positivity or tau positivity in relation to trajectories of memory or EF performance (p -values > 0.11). There were no diagnostic interactions present.

4. Discussion

This article sought to identify and describe interactions between stroke risk factors and AD biomarkers in conferring risk for neurodegeneration and cognitive impairment. Our results suggest that there may be a dynamic interplay between the AD pathological cascade and vascular health, whereby the level of risk for brain

aging associated with vascular risk factors depends in part on the presence or absence of AD pathology.

Stroke risk appears to be most related to brain aging in the absence of AD biomarkers. Although this explanation is somewhat counterintuitive, we observed the effect consistently across phenotypes in ADNI and in the NACC replication sample. The stronger effect of vascular risk in biomarker negative versus positive participants is illustrated in Fig. 1 and suggests that controlling stroke risk factors may be most important in individuals who are otherwise at low risk for AD. Some previous work leveraging neuropathologic measures of cerebrovascular disease and AD have demonstrated a comparable interaction, whereby the effect of cerebrovascular disease was strongest in those participants without AD pathology (Chui et al., 2006). At the same time, we also observed a subtle additive effect at baseline in which the smallest baseline brain volumes and poorest cognitive performances were observed in individuals with high stroke risk and AD biomarker positivity (Fig. 2). The additive effect of AD and stroke risk factors is consistent with previous autopsy findings suggesting an additive effect of cerebrovascular disease on cognition in the presence of AD pathology (Schneider et al., 2007b). Recent work leveraging white-matter hyperintensity and amyloid imaging data has shown a comparable additive effect of amyloid and white-matter hyperintensities on conversion to AD (Provenzano et al., 2013). Our findings, therefore, provide additional evidence that vascular risk and AD biomarkers are independently associated with cognitive impairment, and that each may provide a "hit" that ultimately contributes to the clinical manifestation of dementia.

Recent work by Villeneuve et al. (2014) leveraged a cohort with a wider range of cerebrovascular disease than the participants included here. Their findings suggest the association between stroke risk and parietal cortical thinning may be strongest in amyloid positive participants. We, too, observed some indication of an interactive effect, whereby the most severe brain atrophy was observed in participants with both vascular risk and amyloid positivity (Fig. 2), but our results do not support the conclusion that vascular risk is most predictive in biomarker positive individuals. Compared to the Villeneuve et al. study, our work included a broader representation of the cognitive aging spectrum and a more

restricted range of vascular risk. Thus, it is possible that differences in sample characteristics could explain the disparate findings between prior work and current results. Additional methodological differences potentially underlying the discrepancy includes our evaluation of biomarker levels as a continuous variable in our interaction models, the inclusion of additional covariates (age, sex, education, and diagnosis), the focus on the hippocampus rather than the parietal cortex, the inclusion of more participants in our samples, and our assessment of both cross-sectional and longitudinal changes. Additional work in community and memory clinic referral samples is needed to better understand the dynamic interplay between vascular risk and AD biomarkers across the spectrum of cognitive aging and dementia.

Previous work in ADNI has suggested an independent contribution of cerebrovascular disease (operationalized using white-matter hyperintensities) and CSF-based AD biomarkers to cognitive impairment, however, interactions were not assessed (Barnes et al., 2013). Moreover, white-matter hyperintensities have been associated with decreased glucose metabolism and decline in EF in ADNI but have shown no association with AD biomarker levels (Lo et al., 2012). Again, interactions were not evaluated. Future work focusing on the interactions between regional cerebrovascular disease (e.g., white-matter hyperintensities, cerebral infarcts) and regional AD biomarker load using positron emission tomographic imaging may clarify how vascular risk and injury interacts with AD biomarkers in conferring risk for cognitive decline.

It is interesting that, AD biomarkers showed a stronger relation than stroke risk to all the brain aging outcomes (hippocampal volume, neuropsychological performance) in this analysis, likely due to a selection bias in the ADNI and NACC cohorts. The ADNI enrollment protocol excludes for overt cerebrovascular disease (Hachinski score < 4), so the presence of stroke risk and cerebrovascular pathology is likely under-represented in this population compared with community-based cohorts (Massoud et al., 1999). Despite this limitation, we still observed an association between stroke risk and baseline EF, baseline memory performance, and longitudinal memory performance in ADNI. In all cases, higher stroke risk levels were associated with worse cognitive performance, consistent with previous findings (Brady et al., 2001; Jefferson et al., 2015; Seshadri et al., 2004). Even in a population with a greatly reduced spectrum of cerebrovascular disease, the contribution of adverse vascular health remains important to brain aging.

This article has several strengths including the 2 independent samples that allowed us to extend the observed in vivo interactions between stroke risk and AD biomarkers to ex vivo interactions between vascular and AD pathologies. The inclusion of multiple brain aging phenotypes also allowed for the evaluation of stroke risk and AD biomarkers in the context of both cognition and neurodegeneration. However, this project is not without weaknesses. There may be a bias toward low levels of cerebrovascular disease in the ADNI sample that preclude generalizability to older adults in the population. Relatedly, we also observed smaller effects of vascular health on cognition and brain volume than would be expected from the literature (Elias et al., 2004).

In conclusion, this article identified an interaction between stroke risk factors and AD biomarkers in which the effect of one is strongest in the absence of the other. Future work evaluating these factors in a representative population with more prevalent cerebrovascular disease is warranted to improve the generalizability of the observed interaction effects.

Disclosure statement

The authors have no actual or potential conflicts of interest.

Acknowledgements

This research was supported in part by Pharmaceutical Research and Manufacturers of America Foundation Fellowship in Translational Medicine and Therapeutics (Timothy J. Hohman), Alzheimer's Association IIRG-08-88733 (Angela L. Jefferson), K24-AG046373 (Angela L. Jefferson), R01-AG034962 (Angela L. Jefferson), R01-HL111516 (Angela L. Jefferson), K12-HD043483 (Timothy J. Hohman, Katherine A. Gifford), R01 AG042437 (Shubhabrata Mukherjee), and the Vanderbilt Memory and Alzheimer's Center. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through from generous contributions the following: Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica; Biogen Idec; Bristol-Myers Squibb Company; Eisai; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Medpace; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer; Piramal Imaging; Servier; Synarc; and Takeda Pharmaceutical Company. The Canadian Institutes of Rev December 5, 2013 Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California. The NACC database is funded by NIA/NIH Grant U01 AG016976. NACC data are contributed by the NIA-funded ADCs: P30 AG019610 (PI Eric Reiman, MD), P30 AG013846 (PI Neil Kowall, MD), P50 AG008702 (PI Scott Small, MD), P50 AG025688 (PI Allan Levey, MD, PhD), P30 AG010133 (PI Andrew Saykin, PsyD), P50 AG005146 (PI Marilyn Albert, PhD), P50 AG005134 (PI Bradley Hyman, MD, PhD), P50 AG016574 (PI Ronald Petersen, MD, PhD), P50 AG005138 (PI Mary Sano, PhD), P30 AG008051 (PI Steven Ferris, PhD), P30 AG013854 (PI M. Marsel Mesulam, MD), P30 AG008017 (PI Jeffrey Kaye, MD), P30 AG010161 (PI David Bennett, MD), P30 AG010129 (PI Charles DeCarli, MD), P50 AG016573 (PI Frank LaFerla, PhD), P50 AG016570 (PI David Teplow, PhD), P50 AG005131 (PI Douglas Galasko, MD), P50 AG023501 (PI Bruce Miller, MD), P30 AG035982 (PI Russell Swerdlow, MD), P30 AG028383 (PI Linda Van Eldik, PhD), P30 AG010124 (PI John Trojanowski, MD, PhD), P50 AG005133 (PI Oscar Lopez, MD), P50 AG005142 (PI Helena Chui, MD), P30 AG012300 (PI Roger Rosenberg, MD), P50 AG005136 (PI Thomas Montine, MD, PhD), P50 AG033514 (PI Sanjay Asthana, MD, FRCP), and P50 AG005681 (PI John Morris, MD). The authors appreciate the help of Paul K. Crane, MD MPH and Laura E. Gibbons for providing item parameters for ADNI-Mem and ADNI-EF.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neurobiolaging.2015.05.021>.

References

- Barnes, J., Carmichael, O.T., Leung, K.K., Schwarz, C., Ridgway, G.R., Bartlett, J.W., Biessels, G.J., 2013. Vascular and Alzheimer's disease markers independently predict brain atrophy rate in Alzheimer's Disease Neuroimaging Initiative controls. *Neurobiol. Aging* 34, 1996–2002.
- Beekly, D.L., Ramos, E.M., van Belle, G., Deitrich, W., Clark, A.D., Jack, M.E., Kukull, W.A., 2004. The National Alzheimer's Coordinating Center (NACC) database: an Alzheimer disease database. *Alzheimer Dis. Assoc. Disord.* 18, 270–277.
- Brady, C.B., Spiro, A., McGlinchey-Berroth, R., Milberg, W., Gaziano, J.M., 2001. Stroke risk predicts verbal fluency decline in healthy older men: evidence from the normative aging study. *J. Gerontol. B Psychol. Sci. Soc. Sci.* 56, 340–346.
- Buerger, K., Ewers, M., Andreasen, N., Zinkowski, R., Ishiguro, K., Vanmechelen, E., Hampel, H., 2005. Phosphorylated tau predicts rate of cognitive decline in MCI subjects: a comparative CSF study. *Neurology* 65, 1502–1503.
- Buerger, K., Ewers, M., Pirttil, T., Zinkowski, R., Alafuzoff, I., Teipel, S.J., Soininen, H., 2006. CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease. *Brain* 129, 3035–3041.
- Chui, H.C., Zarow, C., Mack, W.J., Ellis, W.G., Zheng, L., Jagust, W.J., DeCarli, C.C., 2006. Cognitive impact of subcortical vascular and Alzheimer's disease pathology. *Ann. Neurol.* 60, 677–687.
- Crane, P.K., Carle, A., Gibbons, L.E., Insel, P., Mackin, R.S., Gross, A., Harvey, D., 2012. Development and assessment of a composite score for memory in the Alzheimer's Disease Neuroimaging Initiative (ADNI). *Brain Imaging Behav.* 6, 502–516.
- Crane, P.K., Narasimhalu, K., Gibbons, L.E., Mungas, D.M., Haneuse, S., Larson, E.B., van Belle, G., 2008. Item response theory facilitated calibrating cognitive tests and reduced bias in estimated rates of decline. *J. Clin. Epidemiol.* 61, 1018–1027.
- D'Agostino, R.B., Wolf, P.A., Belanger, A.J., Kannel, W.B., 1994. Stroke risk profile: adjustment for antihypertensive medication. The Framingham Study. *Stroke* 25, 40–43.
- Dale, A.M., Fischl, B., Sereno, M.I., 1999. Cortical surface-based analysis: I. Segmentation and surface reconstruction. *Neuroimage* 9, 179–194.
- de Souza, L.C., Chupin, M., Lamari, F., Jardel, C., Leclercq, D., Colliot, O., Sarazin, M., 2012. CSF tau markers are correlated with hippocampal volume in Alzheimer's disease. *Neurobiol. Aging* 33, 1253–1257.
- Desikan, R.S., Segonne, F., Fischl, B., Quinn, B.T., Dickerson, B.C., Blacker, D., Hyman, B.T., 2006. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage* 31, 968–980.
- Diaz-Ruiz, C., Wang, J., Ksiazak-Reding, H., Ho, L., Qian, X., Humala, N., Pasinetti, G., 2009. Role of hypertension in aggravating A β neuropathology of AD type and tau-mediated motor impairment. *Cardiovasc. Psychiatry Neurol.* 2009, 1–9.
- Elias, M.F., Sullivan, L.M., D'Agostino, R.B., Elias, P.K., Beiser, A., Au, R., Wolf, P.A., 2004. Framingham stroke risk profile and lowered cognitive performance. *Stroke* 35, 404–409.
- Fischl, B., Sereno, M.I., Dale, A.M., 1999a. Cortical surface-based analysis: II: inflation, flattening, and a surface-based coordinate system. *Neuroimage* 9, 195–207.
- Fischl, B., Sereno, M.I., Tootell, R.B.H., Dale, A.M., 1999b. High-resolution intersubject averaging and a coordinate system for the cortical surface. *Hum. Brain Mapp.* 8, 272–284.
- Fjell, A.M., Walhovd, K.B., Fennema-Notestine, C., McEvoy, L.K., Hagler, D.J., Holland, D., Dale, A.M., 2010. Brain atrophy in healthy aging is related to CSF levels of Abeta1–42. *Cereb. Cortex* 20, 2069–2079.
- Gentile, M.T., Poulet, R., Pardo, A.D., Cifelli, G., Maffei, A., Vecchione, C., Lembo, G., 2009. β -amyloid deposition in brain is enhanced in mouse models of arterial hypertension. *Neurobiol. Aging* 30, 222–228.
- Gibbons, L.E., Carle, A.C., Mackin, R.S., Harvey, D., Mukherjee, S., Insel, P., Crane, P.K., 2012. A composite score for executive functioning, validated in Alzheimer's Disease Neuroimaging Initiative (ADNI) participants with baseline mild cognitive impairment. *Brain Imaging Behav.* 6, 517–527.
- Jack, C.R., Bernstein, M.A., Fox, N.C., Thompson, P., Alexander, G., Harvey, D., Ward, C., 2008. The Alzheimer's Disease Neuroimaging Initiative (ADNI): MRI methods. *J. Magn. Reson. Imaging* 27, 685–691.
- Jack Jr., C.R., Knopman, D.S., Jagust, W.J., Petersen, R.C., Weiner, M.W., Aisen, P.S., Trojanowski, J.Q., 2013. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol.* 12, 207–216.
- Jack Jr., C.R., Knopman, D.S., Jagust, W.J., Shaw, L.M., Aisen, P.S., Weiner, M.W., Trojanowski, J.Q., 2010. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol.* 9, 119.
- Jagust, W.J., Landau, S.M., Shaw, L.M., Trojanowski, J.Q., Koeppe, R.A., Reiman, E.M., Price, J.C., 2009. Relationships between biomarkers in aging and dementia. *Neurology* 73, 1193–1199.
- Jefferson, A.L., Hohman, T.J., Liu, D., Haj-Hassan, S., Gifford, K.A., Benson, E.M., Ruberg, F.L., 2015. Adverse vascular risk is related to cognitive decline in older adults. *J. Alzheimer's Dis.* 44, 1361–1373.
- Kivipelto, M., Laakso, M.P., Tuomilehto, J., Nissinen, A., Soininen, H., 2002. Hypertension and hypercholesterolaemia as risk factors for Alzheimer's disease. *CNS Drugs* 16, 435–444.
- Lo, R.Y., Jagust, W.J., Weiner, M., Aisen, P., Petersen, R., Jack, C.R., Beckett, L., 2012. Vascular burden and Alzheimer disease pathologic progression. *Neurology* 79, 1349–1355.
- Massoud, F., Devi, G., Stern, Y., Lawton, A., Goldman, J.E., Liu, Y., Mayeux, R., 1999. A clinicopathological comparison of community-based and clinic-based cohorts of patients with dementia. *Arch. Neurol.* 56, 1368–1373.
- Mez, J., Mukherjee, S., Thornton, T., Fardo, D., Trittschuh, E., Sutti, S., Sherva, R., Kauwe, J.S.K., Naj, A., Beecham, G.W., Gross, A., Saykin, A.J., Green, R.C., Crane, P.K. (Under Review). Prominent executive dysfunction in Alzheimer's disease is highly heritable.
- Moreno-Gonzalez, I., Estrada, L.D., Sanchez-Mejias, E., Soto, C., 2013. Smoking exacerbates amyloid pathology in a mouse model of Alzheimer's disease. *Nat. Commun.* 4, 1495.
- 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 beta-amyloid deposition in elderly subjects. *Brain* 132, 1310–1323.
- Muthén, L.K., Muthén, B.O., 1998. *Mplus. Statistical Analyses with Latent Variables. User's Guide*, 3.
- Provenzano, F.A., Muraskin, J., Tosto, G., Narkhede, A., Wasserman, B.T., Griffith, E.Y., Guzman, V.A., Meier, I.B., Zimmerman, M.E., Brickman, A.M., Alzheimer's Disease Neuroimaging Initiative, 2013. White matter hyperintensities and cerebral amyloidosis: necessary and sufficient for clinical expression of Alzheimer disease? *JAMA Neurol.* 70, 455–461.
- Reuter, M., Schmansky, N.J., Rosas, H.D., Fischl, B., 2012. Within-subject template estimation for unbiased longitudinal image analysis. *Neuroimage* 61, 1402–1418.
- Schneider, J.A., Arvanitakis, Z., Bang, W., Bennett, D.A., 2007a. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology* 69, 2197–2204.
- Schneider, J.A., Bennett, D.A., 2010. Where vascular meets neurodegenerative disease. *Stroke* 41 (10 suppl 1), S144–S146.
- Schneider, J.A., Boyle, P.A., Arvanitakis, Z., Bienias, J.L., Bennett, D.A., 2007b. Subcortical infarcts, Alzheimer's disease pathology, and memory function in older persons. *Ann. Neurol.* 62, 59–66.
- Seshadri, S., Wolf, P., Beiser, A., Elias, M., Au, R., Kase, C., DeCarli, C., 2004. Stroke risk profile, brain volume, and cognitive function: the Framingham Offspring Study. *Neurology* 63, 1591–1599.
- Shaw, L.M., Vanderstichele, H., Knapik-Czajka, M., Figurski, M., Coart, E., Blennow, K., Dean, R.A., 2011. Qualification of the analytical and clinical performance of CSF biomarker analyses in ADNI. *Acta Neuropathol.* 121, 597–609.
- Troncoso, J.C., Zonderman, A.B., Resnick, S.M., Crain, B., Pletnikova, O., O'Brien, R.J., 2008. Effect of infarcts on dementia in the Baltimore longitudinal study of aging. *Ann. Neurol.* 64, 168–176.
- Villeneuve, S., Reed, B.R., Madison, C.M., Wirth, M., Marchant, N.L., Kriger, S., Chui, H.C., 2014. Vascular risk and A β interact to reduce cortical thickness in AD vulnerable brain regions. *Neurology* 83, 40–47.
- Wang, L.Y., Larson, E.B., Sonnen, J.A., Shofar, J.B., McCormick, W., Bowen, J.D., Li, G., 2009. Blood pressure and brain injury in older adults: findings from a community-based autopsy study. *J. Am. Geriatr. Soc.* 57, 1975–1981.
- Wolf, P.A., D'Agostino, R.B., Belanger, A.J., Kannel, W.B., 1991. Probability of stroke: a risk profile from the Framingham Study. *Stroke* 22, 312–318.