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TITLE: Blood Biomarker Profile of TBI-Associated Cognitive Impairment Among Old and Young Veterans

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CONTRACTING ORGANIZATION: Northern California Institute for Research and Education

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14. ABSTRACT The goal of this project is to define the biomarker profile of TBI-associated cognitive impairment (TBI-CI) in veterans and compare it to that of veterans with TBI with no CI (TBI), veterans with no TBI and CI (noTBI-CI), and to age-matched controls. Our overall hypothesis is that TBI-associated CI involves a unique biomarker profile that has features distinguishable from CI without TBI and normal aging. Examining biomarkers in exosomes from the central nervous system, we found multiple blood-based biomarkers that differed significantly between the four groups. This study refines our understanding of the underlying mechanisms in TBI-associated CI, helps predict who is at greatest risk of developing CI in veterans with TBI, and identifies who may benefit from interventions and treatment for CI and its prevention.					
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Table of Contents

	<u>Page</u>
Introduction -----	4
Key Words -----	4
Accomplishments -----	4-9
Impact -----	9
Changes/Problems -----	10
Products -----	10
Participants & Other Collaborating Organizations -----	11
Special Reporting Requirements -----	14

Introduction

Military personnel are at high risk for traumatic brain injury (TBI). Two well-recognized and important adverse outcomes of TBI are cognitive impairment (CI) and dementia. While most studies report a 2-3 times increased risk of dementia associated with TBI, the underlying mechanism and type of dementia associated with TBI remains unclear. Some studies link TBI to Alzheimer disease (AD) while others suggest the TBI-associated dementia is more similar to chronic traumatic encephalopathy (CTE). The goal of this project is to define the biomarker profile of TBI-associated CI in veterans and compare it to that of veterans with CI and no TBI history and to controls. Our overall hypothesis is that TBI-associated CI involves a unique biomarker profile that has features distinguishable from CI without TBI and normal aging. Specifically, we hypothesize that: 1) patients with TBI associated CI will have higher phospho-tau/total tau ratio than controls who have not had a TBI, and that 2) TBI-associated CI will be associated with elevations in inflammatory markers compared to controls and 3) a decrease in b-amyloid measures compared to controls but not as low as in the setting of AD. This study will refine our understanding of the underlying mechanisms in TBI-associated CI, help predict who is at greatest risk of developing CI in veterans with TBI, and identify who may benefit from interventions and treatment for CI and its prevention.

Key Words

Traumatic brain injury (TBI), dementia, chronic traumatic encephalopathy (CTE), blood biomarkers, aging, cognitive impairment (CI)

Accomplishments

- **What were the major goals of the project?**
 - Planning, study design, and regulatory approval
 - Study protocols were approved at both sites in the first quarter of the project. The study protocol, measurements and operations manual were completed in the first six months as planned.
 - Identify and enroll older veterans with TBI and normal controls at Armed Forces Retirement Home (AFRH), Washington, DC, and Veterans Home of California-Yountville (VHC-Y), Yountville, CA
 - Data collection is complete. We have data from 57 normal controls and 68 veterans with TBI (35 with CI, and 30 without CI)
 - Enroll veterans with CI but no TBI history at AFRH and VHC-Y
 - Data collection is complete. We have data from 33 veterans with CI but no history of TBI.
 - Identify blood biomarker profile of TBI and compare to that of and controls
 - Across the four groups (Control, noTBI-CI, TBI-noCI, and TBI-CI) we found significant differences in multiple biomarkers. These results have been written into three manuscripts: two under review and one published.

- **What was accomplished under these goals?**

During the entirety of the grant period we worked diligently and met the goals of the project. We collected clinical data and blood from veterans at two different veterans' retirement homes. The blood samples from both sites were sent to Dr. Jessica Gill's lab at NIH for analysis. The preliminary results were presented in a TBI symposium at the Alzheimer's Association International Conference (AAIC) in July of 2018. We then completed a few further analyses and have written up a manuscript, which is currently under review.

We enrolled 155 veterans from two veterans' retirement homes: 90 without TBI and 65 with TBI history. Participants were further separated into CI groups: Controls (no TBI, no CI), n=60; no TBI with CI, n=30; TBI without CI, n=30; and TBI with CI, n=35. TBI was determined by the Ohio State University TBI Identification Method. CI was defined as impaired cognitive testing, dementia diagnosis, or use of dementia medication. Blood specimens were enriched for CNS-derived exosomes. Proteins [neurofilament light (NfL), total tau, glial fibrillary acidic protein (GFAP), alpha synuclein, β -amyloid 42 (A β 42), and phosphorylated tau (pTAU)] and cytokines [tumor necrosis factor alpha (TNFa), interleukin 6 (IL-6) and interleukin 10] were measured using ultrasensitive immunoassays.

Veterans were, on average, 79 years old. In participants with TBI history, 65% had mild TBI; average time from most recent TBI was 37 years. In adjusted analyses, the TBI and CI groups differed on CNS-enriched exosome concentration of pTAU, NfL, IL-6, TNFa (all $p < 0.05$), and GFAP ($p = 0.06$), but not on A β 42 or other markers. Adjusted area under the curve (AUC) analyses found that all significantly associated biomarkers combined separated TBI with/without CI (AUC = 0.85, 95% confidence interval 0.74-0.95) and CI with/without TBI (AUC = 0.88, 95% confidence interval 0.77-0.99).

Overall our study found that increased levels of blood-based, centrally-derived exosomal protein biomarkers associated with TBI-CI can be detected, even decades after TBI. The combined markers differentiated TBI and CI status and consisted of neurodegenerative proteins and inflammatory cytokines. Understanding the etiology of TBI-CI is essential for the development of targeted therapies. (Peltz et al., under review)

Figure 1. Beeswarm Plots for significant biomarkers showing the distribution of all four groups. Overall p-values (adjusted for age and sex) are displayed at the top of each plot. The brackets below show the significant (= $p < 0.05$) pairwise comparisons for each marker.*

Figure 2. ROC curves and AUCs (adjusted for age and sex) for all biomarkers significantly associated with the TBI and CI groups combined (pTAU, NfL, GFAP, IL-6, and TNFa)

Figure 1.

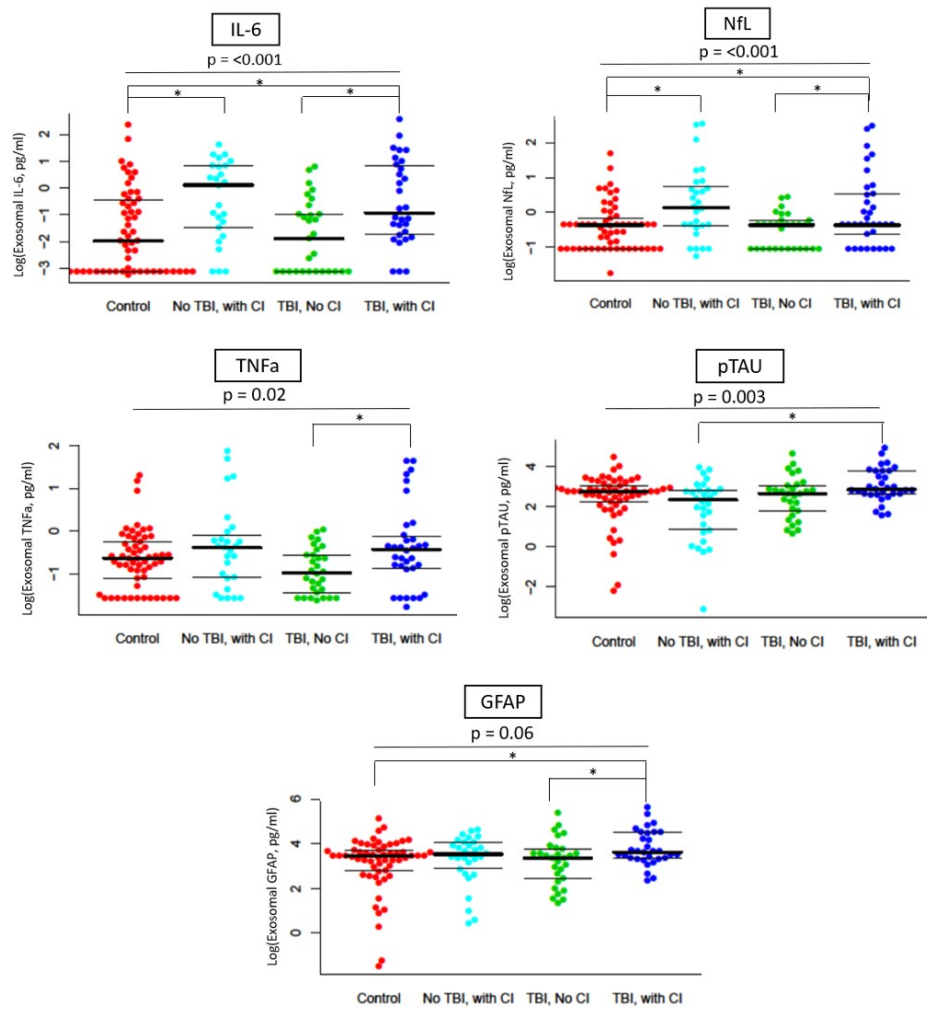
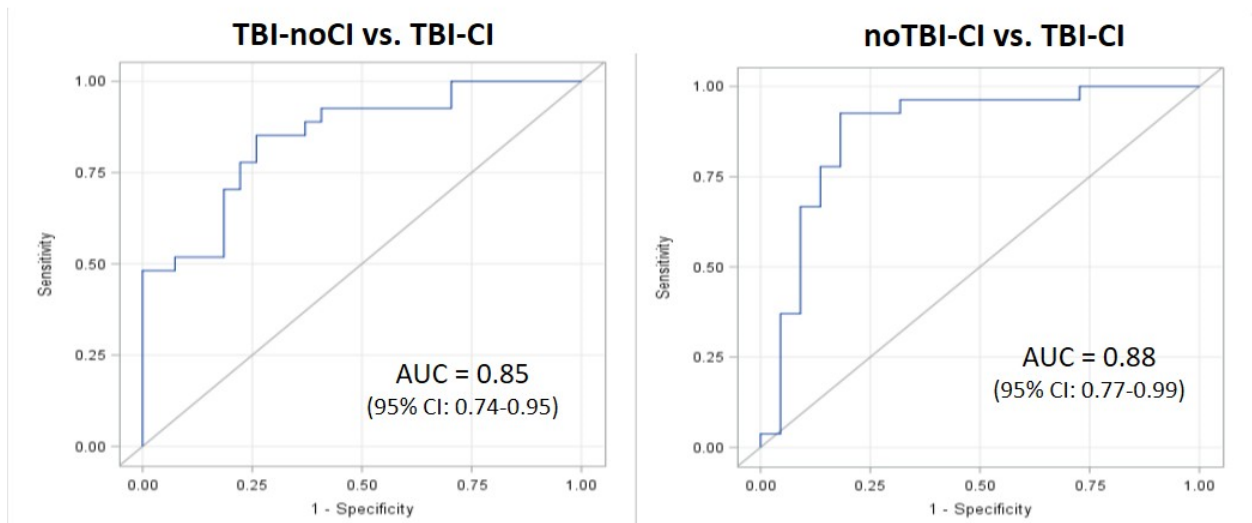


Figure 2.



In collaboration with Dr. Edward Goetzl at UCSF, we used different laboratory methods to examine a subset of participants (only those from the CA site). In the first study (Goetzl et al., 2019) plasmas were examined for four groups of older veterans: no TBI or CI (n=42), no TBI with CI (n=19), TBI without CI (n=21) and TBI with CI (n=26). TBI was sustained 12 to 74 years before study in 75%. NDEs were enriched by sequential precipitation and anti-L1CAM antibody immunoabsorption and extracted protein biomarkers were quantified by enzyme-linked immunosorbent assays.

Chronic NDE biomarkers known to increase for three to 12 months after TBI, including cellular prion protein (PrPc), synaptogyrin-3, P-T181-tau, P-S396-tau, A β 42 and IL-6 were elevated significantly in subjects who had TBI and CI compared to controls with TBI but no CI. Chronic NDE biomarker levels in subjects without TBI showed significantly higher levels of PrPc, synaptogyrin-3, P-T181-tau and A β 42, but not P-S396-tau and IL-6, in those with CI compared to controls without CI. The acute NDE biomarkers claudin-5, annexin VII and aquaporin-4 were not increased in either group with CI. The NDE biomarkers P-S396-tau and IL-6, that are increased distinctively with CI after TBI, may prove useful in evaluating CI in older patients. A β 42 and P-tau species, as well as their respective putative receptors, PrPc and synaptogyrin-3, remain elevated for decades after TBI, and may mediate TBI-associated CI and be useful targets for development of drugs.

Figure 1:

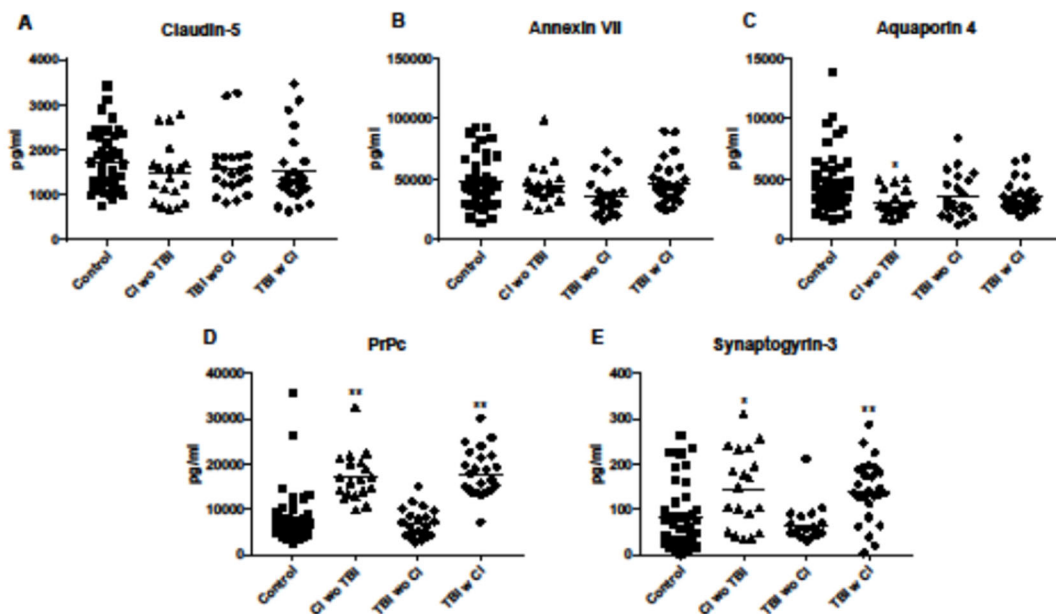
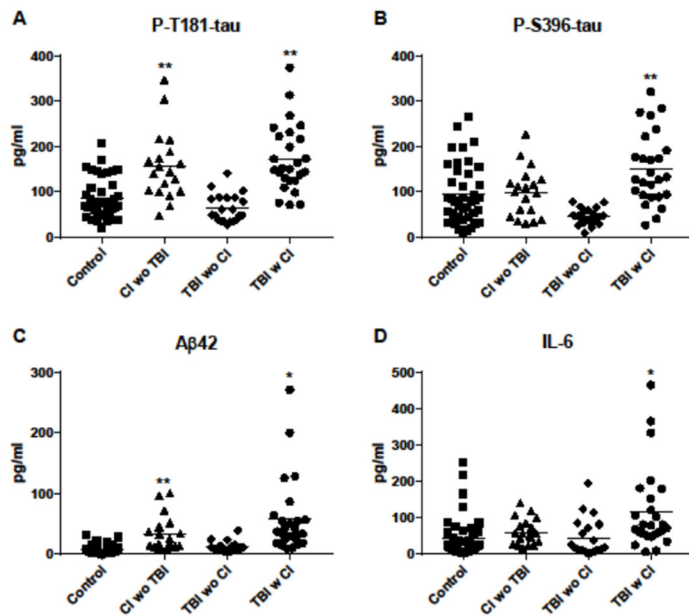
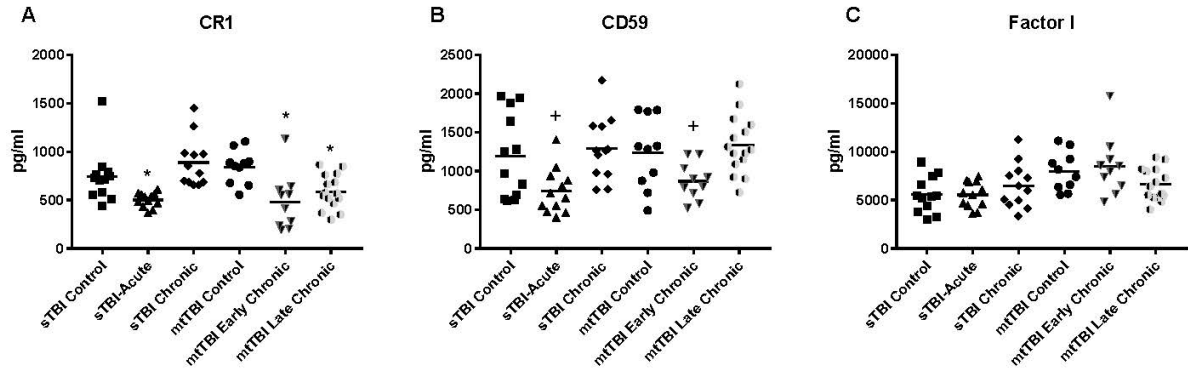


Figure 2:



In a more recent manuscript, a smaller subset of the CA samples from the current project were combined with samples from another study to examine neurotoxic complement proteins in astrocyte-derived exosomes (ADE; Goetzl et al., under review). The study examined sports-related TBI (sTBI) and military-inflicted TBI (mtTBI) subjects without cognitive impairment. We found that plasma levels of ADEs decreased acutely after TBI and returned to normal within months. C protein levels in ADEs were 12- to 35-fold higher than the corresponding levels in neuron-derived exosomes. CD81 exosome marker-normalized ADE levels of classical pathway C4b, alternative pathway factor D and Bb, lectin pathway MBL, and shared neurotoxic effectors C3b and C5b-9 terminal C complex all were significantly higher and those of C regulatory proteins CR1 and CD59 were lower in the first week of acute sTBI (n=12) than in controls (n=12). Most C abnormalities were no longer detected at three to 12 months after sTBI, except for elevated levels of factor D, Bb and MBL. In contrast, significant elevations of ADE levels of C4b, factor D, Bb, MBL, C3b and C5b-9 terminal C complex, and depressions of CR1 and CD59 relative to those of controls were observed one to four years after mtTBI (n=10) and persisted for decades except for abnormalities of Bb, MBL and CD59 (n=15). Complement inhibitors may be useful therapeutically in acute TBI and later in the course of some cases of moderate or severe TBI.

Figure: ADE levels of complement regulatory proteins in sTBI and mtTBI subject groups. Each point represents the value for a control or TBI participant and the horizontal line in point clusters is the mean level for that group. += $p < 0.05$, *= $p < 0.01$.



- **What opportunities for training and professional development has the project provided?**
 - Erica Kornblith, PhD, was a Traumatic Brain Injury/Polytrauma Rehabilitation Research fellow during most of this project. She received training and supervision from Dr. Yaffe on the clinical assessment of TBI and control participants as well as the biomarkers of interest. In December 2019, Dr. Kornblith received a VA Rehabilitation Research and Development Service Career Development Award on the “Rehabilitation of Executive Function in Aging Veterans with History of TBI” (1 IK2 RX003073-01A2).
- **How were the results disseminated to communities of interest?**
 - Preliminary results were presented at an international conference for clinicians and researchers. The final results have been written up in manuscripts that are published or submitted to peer-reviewed journals.
- **What do you plan to do during the next reporting period to accomplish the goals?**
 - Nothing to report

Impact

- **What was the impact on the development of the principal discipline(s) of the project?**
 - This project was one of the first to report that elevations in certain proteins and inflammatory cytokines can be found in the blood of TBI patients more than 25 years post-injury. Future investigations building on these results and examining other markers may help determine the underlying pathology of remote TBI and related CI
- **What was the impact on other disciplines?**
 - Nothing to report
- **What was the impact on technology transfer?**
 - Nothing to report
- **What was the impact on society beyond science and technology?**
 - Nothing to report

Changes/Problems

- **Changes in approach and reasons for change**
 - Nothing to report
- **Actual or anticipated problems or delays and actions or plans to resolve them**
 - As mentioned in previous reports, the AFRH site experienced delays and difficulties in receiving permission to continue to perform research at AFRH. They were unable to resolve these issues and thus, additional recruitment was done at the VHC-Y site. Due to the delay, we requested and received a 2nd Extension Without Funds (EWOFF).
- **Changes that had a significant impact on expenditures**
 - Due to the delay at AFRH, we requested and received a second EWOFF to complete the project as originally designed.
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
 - Nothing to report

Products

- **Publications, conference papers, and presentations**
 - **Journal publications.**

Publications:

1. Goetzl EJ, Peltz CB, Mustapic M, Kapogiannis D, Yaffe K. Neuron-Derived Plasma Exosome Proteins after Remote Traumatic Brain Injury. *J Neurotrauma*. 2019 Oct 11. doi: 10.1089/neu.2019.6711. [Epub ahead of print]
2. Peltz CP, Kenney K, Gill J, Diaz-Arrastia R, Gardner RC, Yaffe K. Blood biomarkers of traumatic brain injury-associated cognitive impairment in older veterans. *Under Review*.
3. Goetzl EJ, Yaffe K, Peltz CB, Ledreux A, Granholm AC, Mustapic M, Kapogiannis D, Tweedie D, Greig N. Traumatic Brain Injury Increases Plasma Astrocyte-Derived Exosome Levels of Neurotoxic Complement Proteins. *Under Review*.

Related Publications:

1. Williams SM, Peltz C, Yaffe K, Schulz P, Sierks MR. CNS Disease Related Protein Variants as Blood-Based Biomarkers in Traumatic Brain Injury. *Neurology*. 2018; 91(15): 702-709.
2. Freimer D, Peltz C, Kaup A, Xia F, Yaffe K. Olfaction as a Marker of Cognitive Impairment in Older Veterans. *Military Behavioral Health*. 2019; 7(1): 118-123.
3. Gardner RC, Peltz CB, Kenney K, Covinsky KE, Diaz-Arrastia R, Yaffe K. Remote traumatic brain injury is associated with motor dysfunction in older military veterans. *J Gerontol Ser A-Biol Sci Med Sci*. 2017; 72(9): 1233-1238.

4. Peltz CB, Gardner RC, Kenney K, Diaz-Arrastia R, Kramer JH, Yaffe K. Neurobehavioral Characteristics of Older Veterans with Remote Traumatic Brain Injury. *J Head Trauma Rehabil.* 2017; 32(1): E8-E15.
5. Kaup AR, Peltz C, Kenney K, Kramer JH, Diaz-Arrastia R, Yaffe K. Neuropsychological Profile of Lifetime Traumatic Brain Injury in Older Veterans. *Journal of the International Neuropsychological Society.* 2017; 23(1):56-64.
6. Kaup AR, Yaffe K. Reassuring News About Football and Cognitive Decline?: Not So Fast. *JAMA Neurology.* 2017; 74(8): 898-899.

- **Books or other non-periodical, one-time publications.** Nothing to report
- **Other publications, conference papers, and presentations.**

1. Yaffe K, Peltz C, Kenney K, Gill J. Blood-Based Biomarker Profile of Older Veterans with Remote TBI. Symposium Presentation at the *2018 Alzheimer’s Association International Conference, Chicago, IL.*
2. Williams SM, Schulz P, Peltz C, Yaffe K, Sierks MR. Neurodegenerative Disease Associated Protein Variants as Potential Blood-Based Biomarkers to Assess Severity of Traumatic Brain Injury. Presented at the *2017 Society for Neuroscience Conference, Washington, DC.*

- **Website(s) or other Internet site(s)**
Nothing to report
- **Technologies or techniques**
Nothing to report
- **Inventions, patent applications, and/or licenses**
Nothing to report
- **Other Products**
Nothing to report

Participants and other collaborating organizations

- **What individuals have worked on the project?**

Name:	<i>Kristine Yaffe</i>
Project Role:	<i>Principal Investigator</i>
Researcher Identifier (e.g. ORCID ID):	<i>KYAFFE</i>
Nearest person month worked:	<i>1</i>
Contribution to Project:	<i>Dr. Yaffe provides leadership and oversees research and data collection at both sites.</i>
Funding Support:	<i>n/a</i>

Name:	<i>Kimbra Kenney</i>
Project Role:	<i>Co-Investigator</i>
Researcher Identifier (e.g. ORCID ID):	<i>KKENNEY</i>
Nearest person month worked:	<i>1 (WOC)</i>
Contribution to Project:	<i>Dr. Kenney provides neurological expertise and oversees the data collection and neurological battery at the AFRH site.</i>
Funding Support:	<i>n/a</i>

Name:	<i>Joel Kramer</i>
Project Role:	<i>Co-Investigator</i>
Researcher Identifier (e.g. ORCID ID):	<i>JKRAMER</i>
Nearest person month worked:	<i>1</i>
Contribution to Project:	<i>Dr. Kramer provides cognitive testing expertise and oversees the neuropsychological testing.</i>
Funding Support:	<i>n/a</i>

Name:	<i>Carrie Peltz</i>
Project Role:	<i>Project Coordinator</i>
Researcher Identifier (e.g. ORCID ID):	<i>n/a</i>
Nearest person month worked:	<i>2</i>
Contribution to Project:	<i>Dr. Peltz coordinates the project at both sites and monitors the day-to-day progress at the VHC-Y site.</i>
Funding Support:	<i>n/a</i>

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Dr. Yaffe:

Summary: Dr. Yaffe had two grants end and two begin in the past year.

End

Title: Change in Sleep & Cognition in Older Women
(Multiple PI: Stone/Yaffe)

Time Commitment: 0.12 calendar months

Supporting Agency: NIH: National Institute on Aging

Performance Period: 10/12 – 03/19NCE

Level of Funding: \$2,068,666 TDC

Title: Alzheimer’s Disease Research Centers: Data and Statistical Core
(Miller/Yaffe)

Time Commitment: 0.12 calendar months

Supporting Agency: NIH: National Institute on Aging

Performance Period: 04/14 – 03/19NCE

Level of Funding: \$528,482 TDC (Core)

Begin

Title: Genetics, comorbidities, and ethnicity: Effects of TBI on Dementia
(Yaffe/Plassman)

Time Commitment: 0.24 calendar months

Supporting Agency: Department of Army

Performance Period: 09/30/18 – 09/29/21

Level of Funding: \$1,299,907 TDC

Title: New Approaches to Dementia Heterogeneity (ADRC)
(Miller)

Time Commitment: 0.12 calendar months

Supporting Agency: NIH: National Institute on Aging

Performance Period: 04/19 – 03/24

Level of Funding: \$12,633,948 TDC

Dr. Kenney:

Summary: Dr. Kenney had no changes in the past year.

Dr. Kramer:

Summary: Dr. Kramer had two grants end and four begin in the past year.

End

Title: Early Age-of-Onset AD: Clinical Heterogeneity and Network Degeneration
(Rabinovici)

Supporting Agency: NIH: National Institute on Aging

Performance Period: 05/01/2014 – 01/31/2020*

Level of Funding: \$450,779

*Dr. Kramer's Role as Co-Investigator has ended on 2/1/2019

Title: Hillblom Network for the Prevention of Age-Associated Cognitive Decline
(Kramer)

Supporting Agency: The Larry L. Hillblom Foundation

Performance Period: 01/01/2015 – 12/31/2018

Level of Funding: \$270,000

Begin

Title: Transseptal Versus Retrograde Aortic Approach to Left Ventricular Catheter Ablation
(Marcus)

Time Commitment: 0.24 calendar months

Supporting Agency: Patient-Centered Outcomes Research Inst

Performance Period: 01/01/2019 – 02/01/2023

Level of Funding: \$688,599

Title: New Approaches to Dementia Heterogeneity (ADRC)
(Kramer)

Time Commitment: 0.54 calendar months

Supporting Agency: NIH: National Institute on Aging

Performance Period: 01/01/2019 – 12/31/2022

Level of Funding: \$12,633,948 TDC

Title: Hillblom Network for the Prevention of Age-Associated Cognitive Decline
(Miller)

Time Commitment: 0.66 calendar months

Supporting Agency: The Larry L. Hillblom Foundation

Performance Period: 04/19 – 03/24

Level of Funding: \$270,000

Title: Immunomodulation to Promote Cerebrovascular and Cognitive Health
(Kramer)

Time Commitment: 0.24 calendar months

Supporting Agency: Stanford University

Performance Period: 01/01/2019 – 12/31/2022

Level of Funding: \$137,500

- **What other organizations were involved as partners?**
 - Nothing to report

Special Reporting Requirements

Nothing to report