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TITLE: Leveraging the Framingham Study to Investigate Relationships Between Traumatic Brain Injury, Military Service, Alzheimer's Disease and Related Dementias

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CONTRACTING ORGANIZATION: Trustees of Boston University, Boston University Medical Center

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6. AUTHOR(S)

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

A large body of evidence suggests that people experiencing a single or repetitive TBI in civilian and military settings may have an increased risk of late-life cognitive decline or neurodegenerative disease, including Alzheimer's disease (AD) and AD-related dementias (ADRD). But the specific clinical features and neuropathological substrates of TBI-associated dementia, as well as the mechanisms underlying this apparent association, are less clear. This project leverages the extensive existing resources of the Framingham Heart Study (FHS), which includes access to a long-committed community-based study sample, as well as health, lifestyle, biomarker, genetic, cognitive, neuroimaging and neuropathological data. We are combining these existing resources with new self-report TBI and military service data. This study will comprehensively characterize the role of TBI and military service on key AD/ADRD outcomes, and identify genetic and non-genetic factors that modify these relationships.

15. SUBJECT TERMS

traumatic brain injury, Alzheimer's disease, dementia, mild cognitive impairment, Parkinson's disease, dementia with Lewy bodies, chronic traumatic encephalopathy, Framingham Heart Study

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

A large body of evidence suggests that people experiencing a single or repetitive TBI in civilian and military settings may have an increased risk of late-life cognitive decline or neurodegenerative disease, including Alzheimer's disease (AD) and AD-related dementias (ADRD). But the specific clinical features and neuropathological substrates of TBI-associated dementia, as well as the mechanisms underlying this apparent association, are less clear. This project leverages the extensive existing resources of the Framingham Heart Study (FHS), which includes access to a long-committed community-based study sample, as well as health, lifestyle, biomarker, genetic, cognitive, neuroimaging and neuropathological data. We are combining these existing resources with new self-report and chart review TBI and military service data. This study will comprehensively characterize the role of TBI and military service on key AD/ADRD outcomes, and identify genetic and non-genetic factors that modify these relationships.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

traumatic brain injury, Alzheimer's disease, dementia, mild cognitive impairment, Parkinson's disease, dementia with Lewy bodies, chronic traumatic encephalopathy, Framingham Heart Study, epidemiology, neuropsychology, neuroimaging, MRI, genetics, neuropathology

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Aims
AIM 1: We will determine the impact of TBI and military service on clinical AD/ADRD outcomes.
AIM 2: We will determine the impact of TBI and military service on AD/ADRD outcomes as measured by structural MRI.
AIM 3: We will determine the impact of TBI and military service on neuropathological AD/ADRD outcomes.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major Task 1: Administrative and Regulatory Tasks: Ongoing (~ 85% complete)

A. Obtain IRB approval for study protocol & FHS data use agreements: complete Y1Q4
B. Seek and obtain approval from U.S. Army Medical Research and Material Command (USAMRMC) Human Research Protection Office (HRPO): complete 10/28/19
C. Submit annual IRB reports and maintain Data Use Agreements (DUA): ongoing
D. Prepare and submit quarterly progress reports to funding agency: ongoing

We worked with the BU IRB, the IMSSM IRB and the USAMRMC HRPO to obtain approvals to conduct the proposed research. Because work on the Framingham Heart Study is longstanding and many BU IRB approvals already existed, but as parts of different BU IRB applications, appropriate documentation for the HRPO took longer than expected. Several iterative changes to the BU IRB protocols were requested by the HRPO and were subsequently made.

Major Task 2: Conduct medical record review: Ongoing (~ 35% complete)

A. Train research staff to conduct medical record review- complete Y1Q4
B. Establish and implement data tracking and quality control protocols – complete Y1Q4
C. Establish and implement TBI review protocol for questionable cases -complete Y1Q4
D. Conduct medical record review for TBI for Gen 2 & Omni Gen (n=5623) - ongoing
E. Conduct on-going data cleaning and integration into FHS database - ongoing
F. Work with FHS to prepare TBI data (Gen 2/OmniGen 1) for sharing with external collaborators -Not started

Prior to approval in Major Task 1, we conducted activities that did not require IRB approval including teaching the research staff how to conduct TBI medical record reviews. We have also implemented a data tracking and quality control protocol and a TBI review protocol for questionable cases so that more clinically experienced reviewers can resolve the questions the research assistant (RA) reviewers may have.

After we received approvals in Major Task 1, we began TBI medical record review. We have access to medical records (including from hospitals, nursing facilities, urgent care, EDs, clinic visits) from nearly all FHS participants. For each TBI, data that results from the medical record review include date of event, time of injury, mechanism of injury, setting of injury, highest level of medical care, time from injury to medical care, duration of hospitalization, clinical signs and symptoms, Glascow Coma Score, ICD codes, presence of, type of and findings from cerebral imaging and confounding variables (such as substance use or previously diagnosed mental illness). Across FHS Gen 2 and Omni cohorts, 1980 charts have been reviewed (790 in the last quarter). As data is collected, it is cleaned and integrated into the FHS database. Chart review had been delayed due covid-19, restricting access to the FHS building, including the chart room. Access to the chart room began again at the end of December 2020.

Major Task 3: Collect self-reported TBI and military data - Timeline: Months 0-36: Ongoing (~ 75% complete)

A. Train staff to oversee administration of self-report questionnaires – complete Y1Q4
B. Establish and implement data tracking and quality control protocols – complete Y1Q4
C. Develop multiple methods for questionnaire administration (RedCap, mail, telephone) – complete Y2Q2

D. Administer self-report questionnaires to living Gen 2 and Omni participants - ongoing
E. ~~Conduct TBI review protocol for questionable cases~~ - included in error, only part of chart review
F. Conduct on-going data cleaning and integration into FHS database - ongoing
G. Work with FHS to prepare TBI data for sharing with external collaborators – not started

Prior to approval in Major Task 1, we conducted activities that did not require IRB approval including teaching the research staff how to oversee the administration of the TBI self-report questionnaires. We have also implemented a data tracking and quality control protocol.

After we received approvals in Major Task 1, we began contacting participants about TBI self-report questionnaires. The questionnaire includes the OSU-TBI-ID to document occurrence of TBI during the course of life, including childhood. For each reported injury, we document type, severity, place, date, cause and mechanisms. It also includes a comprehensive questionnaire regarding military service, contact sports, and experiences that may have resulted in head trauma exposure. Military service questions include branch, years of service, whether combat exposure occurred and TBIs occurring while in the military. Contact sport questions include sport, position, years of play, levels of play and age at first exposure to contact sports. FHS participants who agreed to complete the TBI self-report questionnaire were initially given the option to complete the questionnaire by mail or online. In the mail format, we initially noticed substantial missing dating and have troubleshooted to determine why. We determined that the mailer left too much flexibility for the participants. We have transitioned to the option of online questionnaire or completion over the phone with assistance from an RA. While the data is much improved with the phone call, it is slower and takes more RA resources. We have gone back and called many of the participants who initially completed the mail format. Both options can be completed virtually and continued during the pandemic. During Y3Q3 we made a major push to query most of the remaining Gen 2/Omni1 Cohort about their willingness to participate because we had administered the questionnaire to most who had already consented. We sent a mass email to all remaining living participants who had not yet consented who were agreeable to receive email communication (n=639). We also called cohort members who prefer not to receive email, but are willing to be called (1,279 calls, ~350 participants). Despite this extensive outreach, only 52 questionnaires were completed in Y3Q3, and 10 in Y3Q4, resulting in a total of 1,434 completed questionnaires to date. Please see the problems section below for additional discussion of this issue. In brief, the cohort is being tapped for many FHS projects and only a subset of cohort members are agreeable to participate in this project (most of whom had previously agreed). Although we will make more efforts for recruitment in the coming year when recruitment for other projects has lessened, we do not expect the recruitment numbers to grow much beyond the current level. As data is collected, we will continue to clean and integrate it into the FHS database.

AIM 1: We will determine the impact of TBI and military service on clinical AD/ADRD outcomes.

Major Task 4: Process data sets for proposed analyses on clinical outcomes Timeline: Months 0-24: Ongoing (~ 50% complete)

A. Select variables/request Gen 1 dataset from FHS data staff – completed Y2Q4
B. Merge Gen 1 variables into single dataset and prepare data dictionary – ongoing

C. Quality control steps for Gen 1 including confirming format of ID numbers - ongoing
D. range checks of all data elements to ensure data are within expected ranges; logic checks; consistency of data with published summaries – completed Y3Q4
E. Review data dictionaries and other study documentation for Gen 1 to ensure thorough and complete data request - completed Y3Q4
F. Select variables/request Gen 2/Omni dataset from FHS data staff - not started
G. Use data from medical record review and self-reported TBI to identify cases (those with TBI exposure) and controls (those with no evidence of TBI exposure). Characterize TBI severity using standard criteria. - not started
H. Merge Gen 2/Omni variables into single dataset and prepare data dictionary not started
I. Quality control steps for Gen 2/Omni including confirming format of ID numbers, range checks of all data elements to ensure data are within expected ranges; logic checks; consistency of data with published summaries; - not started
J. Review data dictionaries and other study documentation for Gen 2/Omni to ensure thorough and complete data request -not started

As stated in the grant application, FHS Generation 1 charts had already been reviewed for TBI previous to the grant submission using other resources. For this DOD grant, we proposed to combine this previously collected data with newly collected data from Generation 2. When we began QC and preparation of the Generation 1 dataset, we learned that several charts had been "flagged" by RA reviewers because they had questions that required additional review from more clinically experienced reviewers. This delayed preparation of the Gen 1 dataset. Resolution of flagged Gen 1 charts was ongoing when Covid-19 delays began. Due to the delays, resolution of flagged Gen 1 charts was paused until the end of 12/20 when the chart room reopened. We dedicated substantial effort to resolve the remaining flags in January and February 2021 via MD review. Quality control steps, including logic checks have been completed for this data.
We have requested and received datasets from FHS staff that contain previously collected, non-TBI variables for Gen 1. We have reviewed data dictionaries and datasets to ensure we have all needed variables and correct ID formats, variable ranges and distributions. We have now assembled the full dataset.

Major Task 5: Statistical analyses (marginal effects – aims 1a, b) Timeline: Months 9-30: Ongoing (~ 15% complete)

A. For Gen 1, review/specify details of model design to test the hypotheses that TBI is ~~and military service are independently and jointly~~ associated with risk for MCI, dementia, AD, PD/DLB, decline in cognition, ADLs, mood, and motor function. Note military data is being collected prospectively for Gen 2, but is not available from Gen 1 chart review. We are not obtaining Gen 1 self-report questionnaires as most have passed away. Ongoing.
B. For Gen 1, run statistical models to test the above hypotheses -Ongoing
C. For Gen 1, interpret results of above statistical models - Ongoing
D. For Gen 2/Omni, review/specify details of model design to test the hypotheses that TBI and military service are independently and jointly associated with risk for MCI, dementia, AD and PD/DLB, decline in cognition, ADLs, mood, and motor function. -not started
E. For Gen 2/Omni, run statistical models to test the above hypotheses -not started
F. For Gen 2/Omni, interpret results of above statistical models -not started

We continue to plan models, including choice of covariates. We are focusing on variables that have been shown to be predictive of AD/ADRD outcomes in large epidemiological meta-analyses, including female sex, *APOE* e4, diabetes, smoking status, depression, mid-life hypertension and mid-life obesity. We are currently exploring the data to get a sense of the prevalence and severity of TBI across age and temporal decades. Although we had hoped to use formal ACRM criteria to define TBI in our models, we are finding that a more lenient definition may better capture the true TBI burden. This exploration of the data has convinced us that there will be value in publishing the temporal trends in TBI prevalence, making use of different TBI definitions and testing how TBI is related to mortality in addition to dementia. For the dementia analyses, we have decided that we will construct models in multiple ways. First, we will use the full dataset, treat TBI as time invariant and use time from enrollment as the time variable. Next, will match those with and without TBI by age and sex and use time from TBI as the time variable. We will also conduct stratified analyses by age of TBI.

Major Task 6: Statistical analyses (genetic interactions– aim 1c) Timeline: Months 0-33: Ongoing (~ 18% complete)

A. Review literature for each outcome to identify relevant variants and genes - ongoing
B. Use bioinformatic tools that output related genes and rankings -ongoing
C. Extract data on variants/genes of interest from genome-wide datasets. Note that quality control, imputation and generation of principal components for population substructure has already been completed in previous efforts -not started
D. Conduct gene-based interaction tests for Gen 1 using Aim 1 clinical outcomes -not starte
E. Conduct gene-based interaction tests for Gen 2/Omni using Aim 1 clinical outcomes -not started
F. Interpret results of above genetic models -not started

In preparation for the genetic interaction analyses, we comprehensively reviewed the literature and identified candidate genes and top SNPs and minor allele frequencies for the following phenotypes: poor acute and sub-acute outcomes after TBI, poor chronic outcomes after TBI, Alzheimer's disease, other tauopathies and synucleinopathies. These have been previously added to a table in the appendix. We will periodically review the literature to look for updates until we can begin analyses. We have also identified appropriate bioinformatic tools for gene identification and prioritization after a careful review of available tools. We are using 2 tools: Phenoylzer and MaxLink. Phenoylzer inputs disease and phenotype terms and gene-disease associations and incorporates evidence from PPIs, genetic pathways, gene regulation and functional annotations to output and rank genes. MaxLink inputs seed genes we identified in the literature review and incorporates evidence from PPIs, genetic interactions, genetic regulation, co-expression, and colocalization to output and rank genes. Candidate genes outputted from both of these tools are included in the appendix together with the literature review conducted previously.

Major Task 7: Statistical analyses (demographic, clinical, and lifestyle factors as moderators – aim 1d) Timeline: Months 12-36: Not Started

A. For covariates that demonstrate significant marginal effects in Aims 1a and b for Gen 1, introduce an interaction term between primary exposure and the covariate and also conduct stratified analyses by the primary exposure. -not started

B. For covariates that demonstrate significant marginal effects in Aims 1a and b for Gen 2/Omni, introduce an interaction term between primary exposure and the covariate and also conduct stratified analyses by the primary exposure. -not started
C. Interpret results of above moderation models -not started
D. Prepare Aim 1 results for presentation and publication -not started

AIM 2: We will determine the impact of TBI and military service on AD/ADRD outcomes as measured by structural MRI.

Note that as part of another effort, MRI processing to generate all imaging variables, including harmonized longitudinal data, proposed to use in this grant, is currently being updated using FreeSurfer by colleagues at Harvard. Given the delay in collection of TBI data and the substantial improvement in MRI outcomes with the updated processing, we have not made significant progress on Aim 2. We are expecting to have MRI variables by the end of the calendar year.

Major Task 8: Process data sets for proposed analyses on imaging outcomes Timeline: Months 18-24: Not Started

A. Select imaging variables/request dataset from FHS data staff -not started
B. Merge variables into single dataset that incorporates participants across generations with MRI data and prepare data dictionary- -not started
C. Quality control steps including confirming format of ID numbers, range checks of all data elements to ensure data are within expected ranges; logic checks; consistency of data with published summaries -not started
D. Review data dictionaries and other study documentation to ensure thorough and complete data request -not started

Major Task 9: Statistical analyses (marginal effects – aims 2a, b); Timeline: Months 24-30 Not Started

A. Review/specify details of model design to test the hypotheses that TBI and military service are independently and jointly associated with a) cross- sectionally smaller TCBV, smaller lobar volumes, smaller HV, greater WMHV, decreased FA and increased MD and b) longitudinally greater decline in TCBV and lobar volumes and greater increase in WMHV. -not started
B. Run statistical models to test the above hypotheses -not started
C. Interpret results of above statistical models -not started

Major Task 10: Statistical analyses (genetic interactions – aim 2c) Timeline: Months 0-33; Ongoing (~ 18% complete)

A. Review literature for each outcome to identify relevant variants and genes - ongoing
B. Use bioinformatic tools that output related genes and rankings - ongoing
C. Extract data on variants/genes of interest from genome-wide datasets. Note that quality control, imputation and generation of principal components for population substructure has already been completed in previous effort -not started
D. Conduct gene-based interaction tests using Aim 2 imaging outcomes -not started
E. Interpret results of above genetic models -not started

Progress is the same as Major Task 6.

Major Task 11: Statistical analyses (demographic, clinical, and lifestyle factors as moderators – aim 2d) Timeline: Months 27-36: Not Started

A. For covariates that demonstrate significant marginal effects in Aims 2a and b, introduce an interaction term between primary exposure and the covariate and also conduct stratified analyses by the primary exposure. -not started
B. Interpret results of above moderation models -not started
C. Prepare Aim 2 results for presentation and publication -not started

AIM 3: We will determine the impact of TBI and military service on neuropathological AD/ADRD outcomes.

Major Task 12: Perform quantitation of AP and P-tau in selected regions in FHS brain donors Timeline: Months 0-36: Ongoing (~ 55% complete)

A. Train staff to conduct quantitation—Complete Y1Q4
B. Establish and implement data tracking and quality control protocols – complete Y1Q4
C. Digitally scan slides using Aperio slide scanner - ongoing
D. Label slides with subject ID, region and stain -ongoing
E. Manually circle anatomic regions - ongoing
F. Derive quantitative counts of amyloid and tau stained pixels, amyloid plaques and neurofibrillary tangles - ongoing
G. Conduct on-going data cleaning and integration into FHS database - ongoing
H. Work with FHS to prepare quantitative neuropath data for sharing with external collaborators -not started

As part of other efforts, all brain donors undergo a comprehensive neuropathological exam, including preparation of approximately 75 fixed glass slides across multiple brain regions, using multiple stains. As part of this DOD effort, we are digitally scanning all slides using an Aperio slide scanner.

Prior to approval in Major Task 1, we conducted activities that did not require IRB approval including training staff to conduct quantitation and establishing and implementing data tracking and quality control protocols. Neuropathologists have taught RAs to differentiate gray from white matter, to differentiate subfields of the hippocampus and to identify nuclei like the locus coeruleus. They were also trained on how to use Leica software so that anatomic regions could be manually outlined so that quantitation of pathology can be performed. Values are standardized based on the area outlined and reported as a density. Our data team has built a robust digital tracking system that includes barcoding (indicates ID, region, stain) and tracks individual slides based on current location (as efforts occur at multiple locations), whether scanning has occurred, whether outlining has occurred, whether quantitation has occurred and whether results have been returned to our data team.

Since approval in Major Task 1, we have digitally scanned slides and manually outlined anatomic regions from 147 brain donors (35 this quarter). This effort has been delayed due to covid-19 restrictions (see problems section). BU opened sufficiently that we could restart digital slide scanning in October 2020. Given restrictions on the number of people in a given space and that the

scanner is being used for multiple projects, scanning was slower than prior to the pandemic until February 2021, when an additional scanner was purchased, using separate funds, that has increased scanning capabilities.

Major Task 13: Process data sets for proposed analyses Timeline: Months 18-24: Ongoing (~ 35% complete)

A. Select neuropathology variables/request dataset from FHS data staff – ongoing
B. Merge variables into single dataset that incorporates participants across generations with neuropathology data and prepare data dictionary -ongoing
C. Quality control steps including confirming format of ID numbers, range checks of all data elements to ensure data are within expected ranges; logic checks; consistency of data with published summaries – ongoing
D. Review data dictionaries and other study documentation to ensure thorough and complete data request – ongoing

We have worked with our data team to make sure key neuropathology variables are clean and ready for analysis. This includes CTE diagnosis and stage, Braak stage, CERAD score, Lewy body level (brainstem, transitional, cortical), microinfarcts, macroinfarcts, cerebral amyloid angiopathy, atherosclerosis, arteriolosclerosis and microhemorrhages. Quantitative variables will initially be tau-focused, and include dorsolateral frontal lobe, amygdala, hippocampal subfields and locus coeruleus. The data team is in the process of creating a dataset that incorporates TBI, contact sports, neuropathology and demographic data from the first 150 brain donors whom we have this data on. Because all of the digital slide scan data is not yet ready, we will create the initial dataset with semiquantitative pathology data only and add the digital data when it is ready.

Major Task 14: Statistical analyses (marginal effects – aims 3a, b) Timeline: Months 24-30 (~10% complete)

A. Review/specify details of model design to test the hypotheses that TBI and military service are independently and jointly associated with a) pathologically confirmed AD, PD/DLB and CTE and b) AD/ADRD semi-quantitative (Braak stage, CERAD score, Thal phase, Lewy bodies, TDP-43 and microinfarcts) and quantitative outcomes (average density of p-tau stained pixels and average density of aB stained pixels). -ongoing
B. Run statistical models to test the above hypotheses -not started
C. Interpret results of above statistical models -not started

While the dataset is being prepared, we have discussed model design, including choice of covariates. In these models, demographics, age at death and vascular risk factors will be particularly important. For analyses that use regional burden of tau pathology across multiple correlated regions as outcomes, we will account for the correlation with a linear mixed effects model.

Major Task 15: Statistical analyses (genetic interactions – aim 3c) Timeline: Months 0-36: Ongoing: (~ 18% complete)

A. Review literature for each outcome to identify relevant variants and genes -ongoing
B. Use bioinformatic tools that output related genes and rankings -ongoing

C. Extract data on variants/genes of interest from genome-wide datasets. Note that quality control, imputation and generation of principal components for population substructure has already been completed in previous effort -not started
D. Conduct gene-based interaction tests using Aim 3 neuropathology outcomes -not started
E. Interpret results of above genetic models -not started

Progress is the same as Major Task 6.

Major Task 16: Statistical analyses (demographic, clinical, and lifestyle factors as moderators – aim 3d) Timeline: Months 18-36: Not Started

A. For covariates that demonstrate significant marginal effects in Aims 3a and b, introduce an interaction term between primary exposure and the covariate and also conduct stratified analyses by the primary exposure. -not started
B. Interpret results of above moderation models -not started
C. Prepare Aim 3 results for presentation and publication -not started

Describe the Regulatory Protocol and Activity Status (if applicable).

Describe the Protocol and Activity Status for sections a-c, as applicable, using the format described for each section. If there is nothing significant to report during this reporting period, state "Nothing to Report."

(a) ***Human Use Regulatory Protocols***

TOTAL PROTOCOLS: *State the total number of human use protocols required to complete this project (e.g., 5 human subject research protocols will be required to complete the Statement of Work."). If not applicable, write "No human subjects research will be performed to complete the Statement of Work."*

PROTOCOL(S): *List the identifier and title for all human use protocols needed to complete the project. Include information about the approved target number for clinical significance, type of submission, type of approval with associated dates, and performance status.*

The following format shall be used:

Protocol (of total):

Protocol [HRPO Assigned Number]:

Title:

Target required for clinical significance:

Target approved for clinical significance:

Submitted to and Approved by:

Provide bullet point list of protocol development, submission, amendments, and approvals (include IRB in addition to HRPO).

Status:

Report (i) progress on subject recruitment, screening, enrollment, completion, and numbers of each compared to original planned target(s), e.g., number of subjects enrolled versus total number proposed; (ii) amendments submitted to the IRB and USAMRMC HRPO for review; and (iii) any adverse event/unanticipated problems involving risks to subjects or others and actions or plans for mitigation.

TOTAL PROTOCOLS: 3

PROTOCOL (1 of 3 total):

Protocol [HRPO Assigned Number]: E00206.1a

Title: Leveraging the Framingham Study to Investigate Relationships between Traumatic Brain Injury, Military Service, Alzheimer's Disease and Related Dementias: Prospective

Target required for clinical significance: Although we would like to include as many of the living participants in Gen 2 (2,677) and Omni Gen 1 (433) as possible, our past experience suggests that a realistic goal is to have about 2,400 (~75%) participate in the protocol.

Target approved for clinical significance: N/A

SUBMITTED TO AND APPROVED BY:

- This protocol is currently approved by the Boston University School of Medicine IRB and the USAMRMC HRPO (10/28/19)

STATUS:

(i) Number of subjects recruited/original planned target: N/A
Number of subjects screened/original planned target: N/A
Number of subjects enrolled/original planned target: 1,434/2,400
Number of subjects completed/original planned target: 1,434/2,400

(ii) Report amendments submitted to the IRB and USAMRMC HRPO for review:

- An amendment to this protocol that also incorporates the non-human subjects work (described in the human cadavers section below) is currently approved by the BU SOM IRB and the USAMRMC HRPO
- An amendment to this protocol that changes the language of the consent form to acknowledge that the research is funded by the DOD and that DOD representatives is currently approved by the BU SOM IRB and the USAMRMC HRPO

(iii) Adverse event/unanticipated problems involving risks to subjects or others and actions or plans for mitigation:

None

PROTOCOL (2 of 3 total):

Protocol [HRPO Assigned Number]: E00206.1a

Title: Leveraging the Framingham Study to Investigate Relationships between Traumatic Brain Injury, Military Service, Alzheimer's Disease and Related Dementias: Retrospective

Target required for clinical significance: N/A – all participants are already part of the FHS (Gen 2: 5,124; Omni: 499, Gen 1: 5,209)

Target approved for clinical significance: N/A

SUBMITTED TO AND APPROVED BY:

- This protocol is currently approved by the Boston University School of Medicine IRB and the USAMRMC HRPO (10/28/19)

STATUS:

(i) Number of subjects recruited/original planned target: N/A
Number of subjects screened/original planned target: N/A
Number of subjects enrolled/original planned target: N/A
Number of subjects completed/original planned target: 7,189/10,832

(ii) Report amendments submitted to the IRB and USAMRMC HRPO for review:

- An amendment to this protocol that also incorporates the non-human subjects work (described in the human cadavers section below) is currently approved by the BU SOM IRB and the USAMRMC HRPO

(iii) Adverse event/unanticipated problems involving risks to subjects or others and actions or plans for mitigation:
None

PROTOCOL (3 of 3 total):

Protocol [HRPO Assigned Number]: E00206.1b

Title: Leveraging the Framingham Study to Investigate Relationships between Traumatic Brain Injury, Military Service, Alzheimer's Disease and Related Dementias

Target required for clinical significance: N/A – all participants are already part of the FHS (Gen 1 5,209; Gen 2: 5,124; Omni: 499)

Target approved for clinical significance: N/A

SUBMITTED TO AND APPROVED BY:

- **Note that this protocol is for non-human subjects work only**
- This protocol is currently approved by the Icahn School of Medicine at Mount Sinai IRB and the USAMRMC HRPO

STATUS:

(i) Number of subjects recruited/original planned target: N/A
Number of subjects screened/original planned target: N/A
Number of patients enrolled/original planned target: N/A
Number of patients completed/original planned target: N/A

(ii) Report amendments submitted to the IRB and USAMRMC HRPO for review:
None

(iii) Adverse event/unanticipated problems involving risks to subjects or others and actions or plans for mitigation:
None

(b) Use of Human Cadavers for Research Development Test & Evaluation (RDT&E), Education or Training

"Cadaver" is defined as a deceased person or portion thereof, and is synonymous with the terms "human cadaver" and "post-mortem human subject" or "PMHS." The term includes organs, tissues, eyes, bones, arteries or other specimens obtained from an individual upon or after death. The term "cadaver" does not include portions of an individual person, such as organs, tissue or blood, that were removed while the individual was alive (for example, if a living person donated tissue for use in future research protocols, that

tissue is not considered a "cadaver" under this policy, regardless of whether the donor is living or deceased at the time of tissue use).

***TOTAL ACTIVITIES**: State the total number of RDT&E, education or training activities that will involve cadavers. If not applicable, write "No RDT&E, education or training activities involving human cadavers will be performed to complete the Statement of Work (SOW)."*

***ACTIVITIES:** Provide the following information in a bulleted list for all RDT&E, education or training activities involving human cadavers conducted or supported during the quarter:*

- *Title of the RDT&E, education or training activity*
- *SOW task/aim associated with the activity*
- *Date the activity was conducted*
- *Identification of the organization's responsible individual (e.g., PI or individual primarily responsible for the activity's conduct)*
- *Brief description of the use(s) of cadavers in the activity and the total number of cadavers used during the reporting period*
- *Brief description of the Department of Army organization's involvement in the activity*
- *Status of document submission and approvals*
- *Problems encountered in the procurement, inventory, use, storage, transfer, transportation and disposition of cadavers used for RDT&E, education or training. Examples of problems include but are not limited to: loss of confidentiality of cadaveric donors, breach of security, significant deviation from the approved protocol, failure to comply with state laws and/or institutional policies and public relations issues.*

***TOTAL ACTIVITIES**: 1*

***ACTIVITES**:*

- Title: Digital Slide Scanning and Quantitation
- Major Task 12: Perform quantitation of P-tau in selected regions in FHS brain donors
- This activity is ongoing
- Responsible individual: Jesse Mez
- We are scanning all slides (approximately 75 per case) from each FHS donor (approximately 200). Anatomic regions (gray matter from superior frontal, dorsolateral frontal, inferior frontal, superior temporal, inferior parietal, and calcarine cortices, hippocampus, amygdala, substantia nigra, and locus coeruleus) will be manually outlined so that quantitation of neurofibrillary tangles and stained p-tau pixels can be performed. Values are standardized based on the area outlined and reported as a density.
- Department of Army organization is not involved
- Although this work is not human subjects work, it is currently approved as an amendment to protocols 1 and 2 in the Human Subjects section described above. The amendment is approved by the BU IRB and the DOD HRPO.
- No problems have been encountered

(c) Animal Use Regulatory Protocols

TOTAL PROTOCOL(S):

State the total number of animal use protocols required to complete this project (e.g., 2 animal use research protocols will be required to complete the Statement of Work.). If not applicable, write "No animal use research will be performed to complete the Statement of Work."

PROTOCOL(S):

List the identifier and title for all animal use protocols needed to complete the project. Include information about the approved target number for statistical significance, type of submission, type of approval with associated dates, and performance status.

The following format shall be used:

Protocol (of total):

Protocol [ACURO Assigned Number]:

Title:

Target required for statistical significance:

Target approved for statistical significance:

Submitted to and Approved by:

Provide bullet point list of protocol development, submission, amendments, and approvals (include IACUC in addition to ACURO).

Status:

Provide bullet point list of performance and/or progress status relating to the above protocol and discuss any administrative, technical, or logistical issues that may impact performance or progress of the study (e.g. animal use protocol needs revision to minimize animal suffering, animal protocol modification to include additional staff) for the above ACURO approved protocol.

TOTAL PROTOCOL(S): No animal use research will be performed to complete the Statement of Work

PROTOCOL (of total):

Protocol [ACURO Assigned Number]:

Title:

Target required for statistical significance:

Target approved for statistical significance:

SUBMITTED TO AND APPROVED BY:

STATUS:

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist

others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

During the next quarter, we will continue contacting living Gen 2 and Omni participants for self- and informant report TBI/RHI questionnaires. As we've largely exhausted who is willing to do the self-report, we will focus on the informant report in the next quarter. We will also continue medical record review for TBI for Gen 2 & Omni. Covid building restrictions have been loosened, but there are still more restrictions than prior to Covid, so access to charts and number of people in the space makes progress slower. We will also continue to resolve chart review questions (i.e. flags) for Gen 2 & Omni in the next quarter. We will continue digital slide scanning and quantitation. We will continue to routinely review the literature to identify new genetic loci to potentially include in future genetic analyses. A new AD GWAS was just published in September 2021 and we will these loci next quarter. We will continue to analyze the Gen 1 chart review dataset in the next quarter as described in more detail in major task 5. We plan on having 2 abstracts ready for submission for AAIC in the next quarter. We also plan to complete the preparation of an initial neuropathology dataset in the next quarter.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*

- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Note that this is an ongoing list of problems and resolutions, not just for the past cycle.

Approval of research protocols by the BU IRB and DOD HRPO took 14 months from start of study period. This delayed data collection. This issue is now resolved with the approvals.

As stated in the grant application, FHS Generation 1 charts had already been reviewed for TBI previous to the grant submission using other resources. For this DOD grant, we proposed to combine this previously collected data with newly collected data from Generation 2. When we began QC and preparation of the Generation 1 dataset, we learned that several charts had been "flagged" by RA reviewers because they had questions that required additional review from more clinically experienced reviewers. This delayed preparation of the Gen 1 dataset. We have since identified more experienced reviewers able to assist with reviewing flagged charts. We have also created a protocol for reviewing flagged charts. Resolution of flagged charts was ongoing when Covid-19 delays began. All Generation 1 chart flags were resolved by the end of February 2021.

Due to the Covid-19 pandemic, the FHS office and lab space closed on March 13, 2020 and all employees were restricted from coming to work. Per FHS rules, medical charts can only be reviewed in the FHS space. For this reason, little progress occurred on chart review after the FHS space closed. We worked remotely via teleconference and reassigned some responsibilities so that effort was dedicated to tasks that could be completed virtually. Specifically, contacting living Gen 2 and Omni participants for self-report TBI/RHI questionnaires continued. The FHS space remained closed until late December 2020. Chart review has resumed with reopening. Even with reopening, there are ongoing restrictions related to the number of people in the space concurrently and times for entry and exit, which slows productivity. Similarly, BU office space, where digital slide scanning occurs, closed at the same time, preventing scanning of additional cases. However, outlining anatomic regions on previously scanned slides for quantitation of pathology could be performed remotely and we shifted effort to this front. In Fall 2020, BU opened sufficiently that we could restart digital slide scanning. Given restrictions on the number of people in a given space and that the scanner is being used for multiple projects, scanning remained slower than prior to the pandemic until April 2021.

FHS participants who agreed to complete the TBI self-report questionnaire were initially given the option to complete the questionnaire by paper mail or online. In the mail format, we initially noticed substantial missing dating and have troubleshooted to determine why. We determined that the mailer left too much flexibility for the participants to skip questions. We have transitioned to the option of online questionnaire or completion over the phone with assistance from an RA. While the data is much more complete with the phone call, it is slower and takes more RA resources. This transition was completed in Y2Q3.

As the Covid-19 pandemic has somewhat eased and FHS participants can again be recruited for core evaluations and ancillary studies, like the TBI project, the demands on their time have increased substantially. Because of this, recruitment has recently suffered. We've now reached out to nearly all living FHS Gen 2/Omni participants about participation. We had hoped for about 75% participation (~2400) but our numbers are closer to 1400. We plan to make another recruitment push in the early spring when the Core has finished Gen 2 evaluations and there is less demand for participants' time.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

We delayed planned expenditures in the first 14 months prior to HRPO approval because research protocol approvals had not been completed. For Covid-related delays, as noted above, we reassigned some responsibilities rather than not paying research staff. However, for chart review, we had planned a large effort over the summer of 2020 with approximately 10 summer interns with several of them continuing into the fall and winter to rapidly carry out the chart review. We did not bring any of them on because of the Covid-restrictions. Expenditures have picked up with the relaxation of Covid-restrictions, but we have funds reserved for a year of no cost extension.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

N/A

Significant changes in use of biohazards and/or select agents

N/A

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

We do not yet have results to report. The digital slide images and quantitation we are generating will be a valuable resource for a variety of neuropathology projects that extend beyond TBI-neurodegenerative relationships. We are planning to leverage these data as part of a recently NIH-funded U19 that will explore a range of vascular risk factors and blood based biomarkers that were collected in the FHS in life and their relationship with quantitative neurodegenerative neuropathological outcomes.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*

Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Jesse Mez
Project Role: PI
Researcher Identifier (e.g. ORCID ID): 0000-0003-1438-5442
Nearest person month worked: 0.19 FTE
Contribution to Project: No Change

Name: Kristen Dams-O'Connor
Project Role: PI
Researcher Identifier (e.g. ORCID ID): 0000-0002-2506-0216
Nearest person month worked: .08 FTE
Contribution to Project: No Change

Name: Nicole Saltiel
Project Role: Research Assistant
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 0.5 FTE
Contribution to Project: No Change

Name: Jaeyoon Chung
Project Role: Analyst
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 0.1 FTE
Contribution to Project: No Change

Name: Shruti Durape
Project Role: Research Assistant
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 0.43 FTE
Contribution to Project: No Change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*