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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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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

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TABLE OF CONTENTS

1. INTRODUCTION:

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:

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:

What were the major goals of the project?

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?

Major Task 1: Administrative and Regulatory Tasks: Ongoing (~ 95% 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 (100% 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) – Y4Q3
E. Conduct on-going data cleaning and integration into FHS database – Y4Q3
F. Work with FHS to prepare TBI data (Gen 2/OmniGen 1) for sharing with external collaborators -Y4Q4

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). 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. Across FHS Gen 2 and Omni cohorts, all 5,623 charts have been reviewed. As data was collected, it was cleaned and integrated into the FHS database. We have completed making data dictionaries both for us and for sharing with external collaborators.

Major Task 3: Collect self-reported TBI and military data - Timeline: Months 0-36: Ongoing (100% 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 – completed Y4Q2
E. ~~Conduct TBI review protocol for questionable case~~s - included in error, only part of chart review
F. Conduct on-going data cleaning and integration into FHS database – Y4Q3
G. Work with FHS to prepare TBI data for sharing with external collaborators – Y4Q4

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 will 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). In Y4Q1, we also called 60 informants for participants who lack capacity to consent themselves due to impairment. We made a last push for recruitment in Y4Q2 for both informants and participants for whom we had not previously exhausted contact, resulting in another 23 questionnaires completed. Despite this extensive outreach, only 98 questionnaires were completed since Y3Q3, resulting in a total of 1,470 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). As data were collected, we continued to clean and integrate it into the FHS database. We have completed data cleaning and data dictionary preparation both for us and for sharing with external collaborators.

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 (~ 90% 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 – completed Y4Q1
C. Quality control steps for Gen 1 including confirming format of ID numbers - completed Y4Q1
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 – completed Y4Q3
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. - ongoing
H. Merge Gen 2/Omni variables into single dataset and prepare data dictionary-completed Y4Q4

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; - completed Y4Q4
J. Review data dictionaries and other study documentation for Gen 2/Omni to ensure thorough and complete data request -completed Y4Q4

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. Unfortunately, while we thought this had been completed during Y3Q4, we discovered a discrepancy in an important additional logic check (comparison of chart review TBI with legacy TBI data) which took several weeks of additional chart review to resolve. We have requested and received datasets from FHS staff that contain previously collected, non-TBI variables for Gen 1 and Gen 2/Omni. 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 datasets for Gen 1 retrospective, Gen 2/Omni retrospective and Gen 2/Omni prospective datasets, performed data cleaning and prepared data dictionaries. We have not yet combined Gen 2/Omni retrospective and prospective. This will be for a separate dataset to specifically analyze how retrospective and prospective datasets compare among those we have data on both.

Major Task 5: Statistical analyses (marginal effects – aims 1a, b) Timeline: Months 9-30: Ongoing (~ 65% 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. -Ongoing
E. For Gen 2/Omni, run statistical models to test the above hypotheses -ongoing
F. For Gen 2/Omni, interpret results of above statistical models -ongoing

We continue to plan and run statistical analyses. For 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 previously explored the data to get a sense of the prevalence and severity of TBI across age and temporal decades. We are using ACRM/DOD/VA criteria to define TBI in our models with some modification for mild TBI to 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 and testing how TBI is related to mortality in

addition to dementia. These analyses are ongoing and we presented preliminary results at the American Public Health Association conference. For the dementia and mortality analyses, we are matching those with and without TBI by birth year and sex and using time from TBI as the time variable. For analyses using the retrospective chart review data, we are running analyses across Gen1, Gen2 and Omni combined and running sensitivity analyses stratified by generation. We are also conducting stratified analyses by age of TBI. For the mortality analyses, we are using Fine and Gray competing risk models for the different causes of death. For the dementia analyses, we are using a semi-competing risk model that accounts for death as a competing risk when the outcome of interest is non-terminal. We have preliminary results for several of these analyses using traditional cox-proportional hazards as we continue to refine code for the competing risk models. For the continuous outcomes (cognition, function), we are using mixed effects models with time x group (TBI) interaction with a random slope and intercept. We are interested in different rates of decline by TBI status.

Major Task 6: Statistical analyses (genetic interactions– aim 1c) Timeline: Months 0-33: Ongoing (~ 40% complete)

A. Review literature for each outcome to identify relevant variants and genes – completed Y4Q3
B. Use bioinformatic tools that output related genes and rankings - completed Y4Q3
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 - completed Y4Q3
D. Conduct gene-based interaction tests for Gen 1 using Aim 1 clinical outcomes -ongoing
E. Conduct gene-based interaction tests for Gen 2/Omni using Aim 1 clinical outcomes -ongoing
F. Interpret results of above genetic models -ongoing

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 have added additional SNPs from newly published GWAS. We have also identified appropriate bioinformatic tools for gene identification and prioritization after a careful review of available tools. We have used 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 were previously included in the appendix together with the literature review. We have extracted data on variants/genes of interest from our genome-wide datasets. For these analyses, we are combining Gen 1, Gen 2 and Omni data. Additionally, although estimates for covariates in Gen1 analyses are still preliminary (as are the TBI findings), we have concurrently written code and looked at preliminary findings for stratified analyses by APOE e4 status which was genotyped separately from the GWAS data.

Major Task 7: Statistical analyses (demographic, clinical, and lifestyle factors as moderators – aim 1d) Timeline: Months 12-36: (~ 25% complete)

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. -ongoing

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. -ongoing
C. Interpret results of above moderation models -ongoing
D. Prepare Aim 1 results for presentation and publication -ongoing

Although estimates for covariates are still preliminary (as are the TBI findings), we have concurrently written code and looked at preliminary findings for stratified analyses by sex.

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

In the initial application, we proposed to use MRI data that had been previously processed by another group that was made available to all FHS investigators. Significant problems in the processing of this data have since been identified. To address these issues, as part of another effort, MRI processing to generate all imaging variables, including harmonized longitudinal data, is currently being updated using FreeSurfer by colleagues at Harvard. Given the concerns about the previous MRI data, we have not made significant progress on Aim 2. Our Harvard colleagues hope to have these data to us shortly. However, if we don't receive the updated processing data shortly, we will consider using the previously processed data for cross-sectional analyses that will not be susceptible to the same concerns as the longitudinal data.

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 (~ 30% complete)

A. Review literature for each outcome to identify relevant variants and genes - completed Y4Q3
B. Use bioinformatic tools that output related genes and rankings - completed Y4Q3
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 - completed Y4Q3

D. Conduct gene-based interaction tests using Aim 2 imaging outcomes -not started
E. Interpret results of above genetic models -not started

Progress is similar to Major Task 6 for A, B and C.

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 (~ 95% 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 – complete Y4Q3
D. Label slides with subject ID, region and stain - complete Y4Q3
E. Manually circle anatomic regions - complete Y4Q3
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 245 brain donors, accounting for all available brains and exceeding the 200 we proposed. This effort had 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. We are in the process of deriving the last set of quantitative counts, data cleaning and integration into the FHS database.

Major Task 13: Process data sets for proposed analyses Timeline: Months 18-24: Ongoing (~ 90% complete)

A. Select neuropathology variables/request dataset from FHS data staff – complete Y4Q3
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 are tau-focused, and include dorsolateral frontal lobe, amygdala, hippocampal subfields and locus coeruleus. We have completed creating a dataset that incorporates TBI, contact sports, neuropathology and demographic data from the first 150 brain donors whom we have this data on from another project that called brain donor info. On preparing this dataset, we realized that the TBI and contact sport data that was collected on paper and double entered in our database as part of a separate effort in 2015 had never been previously cleaned or used. We have now cleaned this data by returning to the original paper copies to resolve discrepancies in double entry. We have an updated dataset that is ready for analyses. We are also in the process of combining our chart review TBI data with our neuropathology data, which we have on all brain donors.

Major Task 14: Statistical analyses (marginal effects – aims 3a, b) Timeline: Months 24-30 (~40% 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 -ongoing
C. Interpret results of above statistical models -ongoing

We have specified model design, including choice of covariates. In these models, demographics, age at death and vascular risk factors are particularly important. For analyses that use regional burden of tau pathology across multiple correlated regions as outcomes, we are accounting for the correlation with a linear mixed effects model. We have explored the data with descriptive statistics and run models that we thought would be most novel and well powered for the 150 donors mentioned above. This includes analyses with TBI, contact sport play and quantitative

and semi-quantitative regional tau pathology. Thus far, we have not observed associations. We plan to repeat analyses with the larger chart-review TBI dataset. We are also comparing those demented individuals with TBI compared to those without, to identify differences in pathological etiology of dementia.

Major Task 15: Statistical analyses (genetic interactions – aim 3c) Timeline: Months 0-36: Ongoing: (~ 30% complete)

A. Review literature for each outcome to identify relevant variants and genes - completed Y4Q3
B. Use bioinformatic tools that output related genes and rankings - completed Y4Q3
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 - completed Y4Q3
D. Conduct gene-based interaction tests using Aim 3 neuropathology outcomes -not started
E. Interpret results of above genetic models -not started

Progress is similar to Major Task 6 for A, B and C.

Major Task 16: Statistical analyses (demographic, clinical, and lifestyle factors as moderators – aim 3d) Timeline: Months 18-36: (~5% complete)

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. - ongoing
B. Interpret results of above moderation models -not started
C. Prepare Aim 3 results for presentation and publication -not started

We have started to examine age stratified results, but as we are not thus far seeing significant associations in the analyses from Major Task 14, we think we are likely underpowered for these stratified analyses. We are hopeful that the larger dataset from the chart review may be more fruitful.

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

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

TOTAL PROTOCOLS:

PROTOCOL(S):
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:1,470/2,400
Number of subjects completed/original planned target: 1,470/2,400

(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: 10,832/10,832

(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 240). 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?

Kurtis Chien, a 3rd-year medical student, has played an active role on the project to learn more about head injuries, dementia and epidemiolocal research. He conducted chart reviews for evidence of TBI and has worked with the data, including calculating the lifetime cumulative incidence of TBI. He has created tables and figures. He presented results at the Boston University Medical Student Research Training Program Symposium. He has been a co-author on abstracts and will be a co-author on future manuscripts.

How were the results disseminated to communities of interest?

We reported our results on the incidence of TBI in the Framingham Heart Study (FHS) at the American Public Health Association Conference in Boston in November 2022. We have submitted an abstract to the Alzheimer's Association International Conference reporting the relationship of TBI and dementia in FHS. The meeting is scheduled in July 2023 and the abstract is included in the appendix.

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

The next reporting period will be dedicated to statistical analyses and manuscript preparation. We are nearly finished analyses and are currently preparing a manuscript reporting the TBI incidence and its relationship to long-term mortality in FHS. We have ongoing analyses and are concurrently preparing a manuscript reporting the relationship between TBI and dementia in FHS. We are also leveraging the FHS neuropathology data to investigate whether among those with dementia, those with TBI have different underlying pathology than those without TBI. We also plan analyses investigating the relationship between TBI and cognitive trajectories. We also plan analyses investigating the relationship between TBI and MRI volumetric and white matter outcomes. For all of these analyses we will also examine how age at TBI, sex, APOE and other known genetic risk factors for AD and related dementias may modify the observed relationships with TBI.

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?

Our findings will likely add to a growing body of literature linking TBI to dementia risk. Although some TBIs are not preventable, TBIs related to high-risk activities like contact sports and military service can be mitigated. Our findings could provide the impetus for reconsidering contact sports in young children and for taking extra precautions against TBI during high-risk activities. Further, understanding the true relationship between TBI and dementia can aid in risk prediction with the goal to develop a personalized medicine approach to dementia prediction and treatment.

What was the impact on other disciplines?

Researchers and clinicians who investigate and care for patients with TBI are often interested in the acute and sub-acute outcomes that occur weeks or months after TBI. Alternatively, researchers and clinicians who investigate and care for patients with dementia are less familiar with the variation in TBI exposure (subconcussive vs. mild vs. severe, single vs. repetitive) and how this may differentially impact risk. Our work serves as a bridge between these two related, but often isolated fields.

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

Nothing to Report

What was the impact on society beyond science and technology?

Contact sports are played by millions of people each year. TBI, particularly from contact sports, is a topic regularly discussed by the news media. While it has long been recognized to cause injuries acutely, the chronic effects, including dementia and early death, have only been recently recognized. Despite the risks it poses, football is very much a part of the fabric of American culture. Our findings could inform choices parents make for their children regarding allowing them to play, alter the national conversation about football and what American society should value and could trigger changes to policies regarding youth play and the acceptable allowable risk from an occupational health standpoint.

5. 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

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

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 resumed with reopening. Even with reopening, there were 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 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 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 instead around 1450, even after another recruitment push when the Core had finished Gen 2 evaluations.

As stated in the grant application, TBI and RHI data on brain donors had been previously collected for another effort. On preparing the neuropathology dataset, we realized that this data that was collected on paper and double entered in the neuropathology database, but had never been previously cleaned or used. To resolve discrepancies in double entry, we needed to return to the original paper copies. This slowed down preparation of the neuropathology dataset. We completed preparation of this dataset at the end of Y4Q2.

In the initial application, we proposed to use MRI data that had been previously processed by another group that was made available to all FHS investigators. Significant concerns in the processing of this data have since been identified, including the observation that regional volumes among some participants were growing larger over time and that there was not sufficient documentation to determine how this may have resulted. To address these issues, funded through another large U19 NIH effort, MRIs are being re-processing using FreeSurfer by colleagues at Harvard, including harmonized longitudinal data. This process was delayed due to FHS executive committee concern about insufficient facial masking for deidentification. This was resolved in the fall of 2021 and processing is ongoing, but data have yet to be returned. We are considering using the previously processed data for cross-sectional analyses that will not be susceptible to the same concerns as the longitudinal data, if we don't receive the updated processing data shortly.

Changes that had a significant impact on expenditures

Note that this an ongoing description:

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. Most remaining funds were spent during the first no cost extension year, during which time we completed all delayed data collection and data cleaning. We will use remaining funds to cover analyses and manuscript preparation.

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

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:

- **Publications, conference papers, and presentations**

Durape et al. Incidence and Prevalence of Traumatic Brain Injury in the Framingham Heart Study. American Public Health Association Annual Meeting, Boston, MA, November 7, 2022. Poster included in appendix.

Durape et al. Association of Traumatic Brain Injury, Mild Cognitive Impairment and Dementia in the Framingham Heart Study. 2023 Alzheimer's Association International Conference. *Submitted.* Abstract included in appendix.

Journal publications.

Traumatic Brain Injury Incidence and Association with Long-term Mortality in the Framingham Heart Study. This manuscript will include the comprehensive chart review data from Generations 1 and 2 of the FHS. It will report on the cumulative incidence of TBI as well as the incidence by age-decade and by calendar-decade. We show large increases in incidence above age 80, mainly driven by falls. It will also report on the relationship of TBI with mortality and causes of mortality using a Fine and Gray competing risk model. We find that TBI is associated with long-term mortality, driven by cardiovascular and "other" causes of death. Although the FHS does not report on dementia-related causes of death, we find that dementia is much more common in the "other" group compared with the named causes, suggesting their deaths were dementia-related. We will also report results stratified by age of TBI and by sex (results still pending). Given the uniqueness of the data we collected and that we observe significant associations, we are aiming for a high tier clinical journal, like Lancet Neurology.

Association of Traumatic Brain Injury, Mild Cognitive Impairment and Dementia in the Framingham Heart Study. This manuscript will include the comprehensive chart review data from Generations 1 and 2 of the FHS and report on associations between TBI, MCI and all-cause dementia. To account for the fact that death can be a competing risk for dementia, but that dementia also causes death, we are using a recently published analytic approach called a semi-competing risk model. We show a relationship between TBI and mild cognitive impairment (MCI) and all-cause dementia. Effect estimates are larger for moderate to severe TBI than mild, but associations remain significant in both groups. Interestingly, although there is a relationship between mild TBI and all-cause dementia, the association is null with Alzheimer's disease dementia, suggesting other etiologies. Among brain donors with dementia, we examine the relationship between TBI and neurodegenerative pathologies (results still pending). We will aim to submit to a journal such as JAMA Neurology or Alzheimer's and Dementia.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

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

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 leveraging these data as part of an 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.

The generated TBI data will become part of the data available to all investigators using FHS data. We are separately funded to harmonize the TBI data generated in this project with TBI data from other large epidemiological studies of aging, including the Adult Change in Thought Study, the Memory and Aging Project, the Religious Orders Study and the Minority and Aging Research Study.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

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: .47 FTE
Contribution to Project: No Change

Name: Eden Price
Project Role: Research Assistant
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 1.0 FTE
Contribution to Project: No Change

Name: Shruti Durape
Project Role: Data Manager
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 0.21 FTE
Contribution to Project: No Change

Name: Rebecca Burton
Project Role: Research Assistant
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 0.45 FTE
Contribution to Project: Chart Review

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

Nothing to Report

What other organizations were involved as partners?

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

QUAD Chart is attached.

8. APPENDICES:

1. Poster from American Public Health Association Annual Meeting
2. Abstract submitted to Alzheimer's Association International Conference