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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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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 Officer 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 <u>this quarterly reporting period only</u> 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.

Major Task 1: Administrative and Regulatory Tasks: Ongoing (100% 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): Y5Q4
D. Prepare and submit quarterly progress reports to funding agency:Y5Q4

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. With this report, all administrative and regulatory tasks will be complete

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 (100% 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. – Y5Q4
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 completed combining Gen 2/Omni retrospective and prospective. This is 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 (~ 90% 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. Completed for dichotomous outcomes. Ongoing for continuous outcomes.
B. For Gen 1, run statistical models to test the above hypotheses - Completed for dichotomous outcomes. Ongoing for continuous outcomes.
C. For Gen 1, interpret results of above statistical models - Completed for dichotomous outcomes. Ongoing for continuous outcomes.
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. - Completed for dichotomous outcomes. Ongoing for continuous outcomes.
E. For Gen 2/Omni, run statistical models to test the above hypotheses - Completed for dichotomous outcomes. Ongoing for continuous outcomes.
F. For Gen 2/Omni, interpret results of above statistical models - Completed for dichotomous outcomes. Ongoing for continuous outcomes.

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 is value in publishing the temporal trends in TBI incidence and testing how TBI is related to mortality in addition to dementia. We presented incidence results at the 2022 American Public Health Association conference. For the dementia and mortality analyses, we have matched those with and without TBI (1:3) by birth year, sex and cohort and use time from TBI as the time variable. For covariates, we have included variables that have been shown to be predictive of AD/ADRD outcomes in large epidemiological meta-analyses, including education, diabetes status, hypertension status, smoking status, cardiovascular disease history and stroke history. For analyses using the retrospective chart review data, we ran analyses across Gen1 and Gen2 combined and ran sensitivity analyses stratified by generation. For the mortality analyses, we ran cause-specific competing risk models for the different causes of death. We find strong relationships with all-cause mortality, with much of the effect driven by dementia-related mortality. Effects are present for TBI both before and after age 60, with stronger effects for moderate to severe TBI. We also see stronger effects for multiple TBIs, compared to a single TBI. Analyses are complete and a manuscript is near completion. 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. This was a difficult model to run, had problems with the software package but were ultimately able to successfully change the approach. We see a relationship with MCI and dementia nearly entirely driven by TBI later in life. We see stronger effects for moderate to severe TBI compared to mild and for multiple TBIs, compared to a single TBI. We have presented these results across multiple iterations at the 2023 American

Academy of Neurology meeting and the 2023 Alzheimer's Association International Conference and a manuscript. Analyses are complete and a manuscript is in preparation. 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. These analyses are ongoing and will continue through other funded efforts.

Major Task 6: Statistical analyses (genetic interactions– aim 1c) Timeline: Months 0-33: Ongoing (~ 100% 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 -Y5Q4
E. Conduct gene-based interaction tests for Gen 2/Omni using Aim 1 clinical outcomes -Y5Q4
F. Interpret results of above genetic models - Y5Q4

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 reviewed these findings and genetic risk in CTE more broadly in a review manuscript we published in 2020 in Seminars in Neurology (see Appendix). 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 are included in the appendix. We have extracted data on variants/genes of interest from our genome-wide datasets. We've run analyses in these genes as well as genome wide using AD as an outcome. Unfortunately, no candidates reached significance after correction for multiple testing. Two loci (within CTNND2, upstream of OLIG3) were not in candidate genes but are potentially interesting if replicated. CTNND2 is expressed in cortex and may be involved in neuronal cell adhesion. OLIG3 is predicted to be involved in neuron differentiation and regulation of transcription. See appendix for Manhattan and QQ plot. Given that we are likely underpowered when only using FHS, we have now acquired TBI data across many of the Alzheimer's Disease Genetic Consortium datasets (n~20,000) in addition to FHS and are repeating these analyses.

Major Task 7: Statistical analyses (demographic, clinical, and lifestyle factors as moderators – aim 1d) Timeline: Months 12-36: (~ 80% 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. - Completed for dichotomous outcomes. Ongoing for continuous outcomes.
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. - Completed for dichotomous outcomes. Ongoing for continuous outcomes.
C. Interpret results of above moderation models - Completed for dichotomous outcomes. Ongoing for continuous outcomes.
D. Prepare Aim 1 results for presentation and publication -ongoing

As noted in Major Task 5, we see larger effects for later age of TBI (specifically stratified by median age of 60) and more than 1 TBI. We also conducted stratified analyses for dementia-related

outcomes for sex, education, diabetes status, hypertension status, smoking status, cardiovascular disease history and stroke history in both Gen 1, Gen 2 and combined. We have not observed differences in effect for the other variables mentioned. As mentioned for Major task 5, we will repeat these analyses for the continuous outcomes once our modeling is completed.

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, such that we only trust cross-sectional, but not longitudinal data. 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. Our Harvard colleagues are unfortunately processing at a rate that is too slow to move forward with our TBI analyses. We ultimately decided to use 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: (100% complete)

A. Select imaging variables/request dataset from FHS data staff -Y5Q3
B. Merge variables into single dataset that incorporates participants across generations with MRI data and prepare data dictionary- Y5Q3
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 -Y5Q3
D. Review data dictionaries and other study documentation to ensure thorough and complete data request -Y5Q3

All data processing steps were completed by Y5Q3.

Major Task 9: Statistical analyses (marginal effects – aims 2a, b); Timeline: Months 24-30: (90% complete)

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~~. -completed Y5Q4
B. Run statistical models to test the above hypotheses -ongoing
C. Interpret results of above statistical models -ongoing

We have specified our cross-sectional models for volumetric and white matter analyses. Due to the MRI issues noted above, we reviewed the distribution of when MRIs were obtained. We decided to limit MRIs to those that were obtained between 2013 and 2018 so that they would be obtained on the same, highest quality machine with the same settings, when participants were as old as possible. Similar to our models for the clinical outcomes, we used a matched 4:1 design of no TBI to TBI with matching on year of birth, sex and cohort. We use traditional linear regression with models further adjusted for total intracranial volume, education, diabetes status, hypertension status, smoking status, cardiovascular disease history and stroke history. We did not find any associations for the main analyses. We have preliminarily repeated the analyses stratified by whether the TBI occurred more than or less than 10 years prior to MRI. We found that TBI prior to 10 years is nominally associated with hippocampal atrophy which does not survive multiple testing correction. These results are very recent and further confirmation that the modeling is correct is ongoing.

Major Task 10: Statistical analyses (genetic interactions – aim 2c) Timeline: Months 0-33; Ongoing (~ 75% 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 -ongoing
E. Interpret results of above genetic models -ongoing

We have conducted the above main analyses stratified by APOE e4 status and similarly do not see differences in associations. We will repeat these analyses stratified by when the TBI occurred (more or less than 10 years prior to MRI). Given the MRI limitations and the lack of associations observed above, we are skeptical that further genetic analyses will be fruitful until our sample size grows. Fortunately, the processing of MRIs using Freesurfer by our Harvard colleagues is moving forward and longitudinal scans processed together should be available in the next year. Additionally the Alzheimer's Disease Sequencing Project Phenotype Harmonization Consortium has recently produced harmonized phenotypes for volumetric and white matter hyperintensity MRI outcomes. We have obtained TBI data as mentioned in Major Task 6 across the ADGC/ADSP and will be able to look at MRI outcomes in addition to AD diagnosis.

Major Task 11: Statistical analyses (demographic, clinical, and lifestyle factors as moderators – aim 2d) Timeline: Months 27-36: (~ 75% complete)

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. - ongoing
B. Interpret results of above moderation models -ongoing
C. Prepare Aim 2 results for presentation and publication -ongoing

Similar to our analyses for clinical outcomes, we have also tested associations stratified by TBI severity (absent vs. mild vs. moderate/severe) and by TBI count (none vs. one vs. more than one). We do not see differences likely because we are underpowered. Similarly we do not see differences when analyses are stratified by sex, education or vascular risk factors. As noted for the genetic analyses, we will be able to increase our sample size in the future which should allow for better powered analyses of effect moderators.

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 – Y5Q1
G. Conduct on-going data cleaning and integration into FHS database – Y5Q4
H. Work with FHS to prepare quantitative neuropath data for sharing with external collaborators -ongoing

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 digitally scanned 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 have derived the last set of quantitative counts. Data cleaning and integration into the FHS database is complete, but sharing is still being worked out due to size and complexity of the data.

Major Task 13: Process data sets for proposed analyses Timeline: Months 18-24: Ongoing (~ 100% 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 -Y5Q3
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 – Y5Q3
D. Review data dictionaries and other study documentation to ensure thorough and complete data request – Y5Q3

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 to collect this 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 have also completed 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 (~85% 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). -Y5Q4
B. Run statistical models to test the above hypotheses -ongoing
C. Interpret results of above statistical models -ongoing

We specified model design, including choice of covariates. In these models, we tried to balance including appropriate covariates to reduce confounding, but limit the total number of covariates given our sample size and limited power. All models include sex and age at death, with sensitivity analyses including vascular risk factors. We have run analyses with TBI, contact sport play, quantitative and semi-quantitative regional tau pathology, CERAD score and Thal phase. Analyses with TDP-43, Lewy bodies and vascular outcomes are still ongoing. For analyses that use regional burden of tau pathology across multiple correlated regions as outcomes, we accounted for the correlation with a linear mixed effects model. Thus far, we observe non-significant trends with quantitative outcomes, including when we use the larger chart-review TBI dataset. Likely, we are still underpowered. Even though we do not see significant relationships, we will include these results in the full TBI-dementia paper, described in Major Task 5. Also, of note, we see CTE in only 2 cases from the FHS brain bank. The frequency of CTE in the general population is still poorly documented and careful characterization of RHI history and clinical course while living is limited. Therefore, we are also working on a descriptive manuscript of the RHI and TBI history of all of the brain donors with case reports of the 2 with CTE. Lastly, given that we are likely underpowered, we are in the process of expanding our analysis to include other community brain banks, including ROS/MAP and ACT to be better powered to find associations.

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. Given the sample size, lack of significant associations for marginal effects and the lack of genetic interactions observed in the much larger clinical sample, we think discovery analyses limited to just FHS brain donors will not be fruitful, but rather could be used as validation after discovery in the larger clinical datasets, particularly after we combine with additional datasets.

Major Task 16: Statistical analyses (demographic, clinical, and lifestyle factors as moderators – aim 3d) Timeline: Months 18-36: (~50% 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 -ongoing
C. Prepare Aim 3 results for presentation and publication -ongoing

In age and sex stratified analyses with TBI, contact sport play, quantitative and semi-quantitative regional tau pathology, CERAD score and Thal phase, we have not seen associations. As noted above, sample size and power are a substantial limitation. We are hopeful that analyses combined with other samples will prove more fruitful.

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. The USAMRMC HRPO (approval 10/28/19) closed this protocol at the end of the grant.

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. The USAMRMC HRPO which had approved this protocol, closed it at the end of the grant.
- An amendment to this protocol changed the language of the consent form to acknowledge that the research is funded by the DOD. It is currently approved by the BU SOM IRB. The USAMRMC HRPO closed the protocol at the end of the grant.

(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. The USAMRMC HRPO (10/28/19) closed this protocol at the end of the grant.

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. The USAMRMC HRPO closed this protocol at the end of the grant.

(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. The USAMRMC HRPO closed this protocol at the end of the grant.

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. The DOD HRPO closed this protocol at the end of the grant.
- 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.

Kurtis Chien, a 4th-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.

Shruti Durape is a foreign medical graduate who completed her MPH at BU and has since worked as a data manager. She has been mentored by a team of clinicians, statisticians and senior data managers and has developed a skill set across all of these spaces. She has led several of the analyses and has been a crucial team member.

Rebecca Burton is a former research assistant and current Master's student who has been involved with the project since the onset and had a large role in conducting and overseeing the

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.

We reported our results on the incidence of TBI in the Framingham Heart Study (FHS) at the 2022 American Public Health Association Conference in Boston. We reported results on the relationship between TBI, dementia and related outcomes across multiple iterations and analyses at the 2023 American Academy of Neurology Annual Meeting in Boston and the 2023 Alzheimer's Association International Conference in Amsterdam. Data that was collected as part of this grant was used to conduct analyses on ice hockey play and risk for CTE and presented at the 2022 American Academy of Neurology Annual Meeting in Seattle.

Statement of future directions and next steps.
An NIH R01 grant (Leveraging Existing Aging Research Networks to investigate TBI and AD/ADRD risk (LEARN TBI & AD), R01AG061028, Total funding:) has been funded to harmonize Framingham TBI and RHI data with TBI and RHI data from the Adult Change in Thought (ACT) study in Seattle and the Religious Orders Study (ROS), Memory and Aging Project (MAP), and the Minority Aging Research Study (MARS) in Chicago. As noted in the major tasks, some of the proposed analyses did not show significant associations, but are likely underpowered. We are reluctant to report these findings as null because we aren't sure if there is a true lack of association or if we are underpowered to find the relationship. This R01 grant will help to resolve this issue, particularly for genetic and neuropathological analyses. This completed DOD application focused on FHS Gen 1 and Gen 2, but did not include Gen 3. We plan to submit a new grant application to conduct similar chart review for TBI of the Gen 3 participants and to continue to collect prospective TBI and RHI data across all generations. This will likely be part of the renewal for R01AG061028.

We have also leveraged the digital slide scan data for a new R01 application that was submitted in November 2023 (Digital Neurodegenerative Pathology after Repetitive Head Impacts, R01AG089465, Total costs:). This application proposes to use digital images from Framingham as well as from several other brain banks to investigate the relationships between repetitive head impacts and neuropathological and clinical outcomes using machine learning to identify patterns of pathology on the digital slides.

Lead candidate product
Knowledge product: TBI is incredibly heterogeneous, from thousands of sub concussive injuries that do not show symptoms acutely to immediately life-altering severe brain injury. At what age the head injuries occur, how many have occurred, the mechanism by which they occurred and the background and comorbidities of the people in whom they have occurred can lead to many different long-term outcomes, including death, dementia and it's neuropathological substrates. Our findings provide important added context regarding the implications of this heterogeneity. For example playing 3-4 years of high-school contact sports may pose low risk for long-term neurodegenerative outcomes, but with the addition of youth or college play, increasing the cumulative burden to 5-10 years, may pose substantial risk. Alternatively, a single moderate TBI in young adulthood from which the person fully recovers, may not pose substantial long-term consequences. However, a similar injury to a 60 year old could have serious implications for dementia and mortality. This work helps to disentangle the implications of this heterogeneity and may prove crucial for a personalized medicine approach to risk prediction, primary prevention, diagnosis and treatment.

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).

Our findings 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. Our findings also suggest that there is a close relationship between falls late in life, traumatic brain injury, dementia and earlier mortality. Although the causal pathway is not entirely clear, prevention of late-life falls could potentially have large effects on reduction of dementia incidence and mortality.

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.

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:

- *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.*

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. Further military service and contact sports are closely intertwined, with most veterans having also participated in contact sports either during childhood and adolescence or while serving. For instance, the military academies all still require boxing. Despite the risks it poses, contact sports are 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 contact sports 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: The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report

Actual Problems or delays and actions to resolve them
Provide a description of current problems or issues that may impede performance or progress of this project along with proposed corrective action. Also 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 t less cost than anticipated. For an award that includes the recruitment of human subjects for clinical research or a clinical trial, discuss any problems or barriers encountered, if applicable, and what has been done to mitigate those issues. Discussion may highlight enrollment problems, retention problems, and actions taken to increase enrollment and/or improve retention.

Note that this is a full list of problems and resolutions.

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.

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. 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 used remaining funds to cover analyses and manuscript preparation in Y5.

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 are no products to report for the current quarter, state "Nothing to report."*

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

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 American Academy of Neurology Annual Conference. Oral Presentation. Slides included in the appendix.

Durape et al. Association of Traumatic Brain Injury, Mild Cognitive Impairment and Dementia in the Framingham Heart Study. Accepted abstract for Alzheimer's Association International Conference, Amsterdam, Netherlands, July 2023. Poster included in the appendix.

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).

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 Cause-specific competing risk model. We find that TBI is associated with long-term mortality, driven by "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 also report results stratified by age of TBI, with findings present both for TBIs that occurred before age 60 and those occurring after age 60, but with stronger effects after age 60. We also see stronger effects for moderate to severe TBI and for multiple TBIs. 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. This is in the late stages of preparation.

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 conducting sensitivity analyses that separately examine relationships between TBI and dementia among people who are still alive or who died after dementia. We show a relationship between TBI, and mild cognitive impairment (MCI) and all-cause dementia and AD-dementia. Effect estimates are larger for moderate to severe TBI than mild, but associations remain significant in both groups. Effects are largely driven by later life TBI (after age 60). Among brain donors with and without TBI, we also present neurodegenerative pathologies. We will aim to submit to a journal such as JAMA Neurology or Alzheimer's and Dementia. This is in intermediate stages of preparation.

Abdolmohammadi B, Dupre A, Evers L, Mez J. Genetics of Chronic Traumatic Encephalopathy. Semin Neurol [Internet]. Thieme Medical Publishers; 2020 Jul 26 [cited 2020 Jul 29]; Available from: http://www.thieme.connect.de/DOI/DOI?10.1055/s-0040-1713631. This is published and included in the appendix.

Abdolmohammadi B. et al. Duration of Ice Hockey and Chronic Traumatic Encephalopathy. This is currently circulated to authors for review and the draft is included in the appendix. We are submitting to Lancet Neurology.

Price E. et al. Repetitive head impact exposure and chronic traumatic encephalopathy in a community brain bank. This is in the early stages of preparation. we see CTE in only 2 cases from the FHS brain bank. The frequency of CTE in the general population is still poorly documented and careful characterization of RHI history and clinical course while living is limited. Therefore, we are also working on a descriptive manuscript of the RHI and TBI history of all of the brain donors with case reports of the 2 with CTE.

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.*

The digital slide images and quantitation we have generated 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. As noted above, they are foundational for an NIH R01 application that we recently submitted to use machine learning to evaluate TBI, RHI, clinical and 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.

The findings from the hockey paper (specifically the cut-points for years of play and risk for CTE) will likely be incorporated into future Traumatic Encephalopathy Syndrome Criteria, used to diagnose CTE in life.

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?
Provide the following information for: (1) Project Directors (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).

Provide the name and identify the role the person played in the project. Indicate the nearest whole person month (Calendar, Academic, Summer) that the individual worked on the project. Show the most senior role in which the person worked on the project for any significant length of time. For example, if an undergraduate student graduated, entered graduate school, and continued to work on the project, show that person as a graduate student, preferably explaining the change in involvement.

Describe how this person contributed to the project. 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.*

All team members who contributed at least 1 person month/yr for at least 1 year. For those who contributed across multiple years, contribution is averaged such that contribution is still “per year”.

Name:	Jesse Mez
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-1438-5442
Nearest person month worked:	2
Contribution to Project:	Oversee team, project conceptualization

Name:	Kristen Dams-O’Connor
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-2506-0216
Nearest person month worked:	1
Contribution to Project:	project conceptualization and oversite

Name:	Shruti Durape
Project Role:	Data Manager
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	6
Contribution to Project:	Data management and analysis

Name:	Nicole Saltiel
Project Role:	Research Assistant
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	6
Contribution to Project:	Digital slide scanning preparation

Name:	Corina Mangione
Project Role:	Research Assistant
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	3
Contribution to Project:	Data quality control, chart review

Name:	Jaeyoon Chung
Project Role:	Analyst
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	1
Contribution to Project:	Genetic analyses

Name:	Rebecca Burton
Project Role:	Research Assistant
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	5
Contribution to Project:	Chart Review

Name:	Eden Price
Project Role:	Research Assistant
Researcher Identifier (e.g. ORCID ID):	6
Nearest person month worked:	1.0 FTE
Contribution to Project:	Chart Review

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.

Nothing to Report

8. Special Reporting Requirements:

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

Please see attachment

***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.*

{{Manuscripts, publications, abstracts, and additional data should be included here. Be sure to organize and reference the appendices in the report as appropriate.}

The following are included as attachments:

1. Quad Chart
2. Poster: 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.
3. Slides from oral presentation: Durape et al. Association of Traumatic Brain Injury, Mild Cognitive Impairment and Dementia in the Framingham Heart Study. 2023 American Academy of Neurology Annual Conference.
4. Poster: Durape et al. Association of Traumatic Brain Injury, Mild Cognitive Impairment and Dementia in the Framingham Heart Study. Accepted abstract for Alzheimer's Association International Conference, Amsterdam, Netherlands, July 2023.
5. Abstract: Durape et al. Association of mid- vs. late-life traumatic brain injury with dementia: the Framingham Heart Study. Upcoming AD/PD Meeting 2024
6. Published manuscript: Abdolmohammadi B, Dupre A, Evers L, Mez J. Genetics of Chronic Traumatic Encephalopathy. Semin Neurol [Internet]. Thieme Medical Publishers; 2020 Jul 26.
7. Manuscript nearly ready for submission: Abdolmohammadi B. et al. Duration of Ice Hockey and Chronic Traumatic Encephalopathy.
8. Gene and SNP table to determine candidate genes
9. Select genetic results
10. Select neuropathology results
11. Select preliminary MRI results