AWARD NUMBER: W81XWH-20-1-0236

TITLE: Validating Novel Brain Imaging Biomarkers for Classifying Mild Traumatic Brain Injury and Subsequent Risks of Alzheimer's Disease in Gulf War Veterans

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REPORT DATE: May 2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE
May 2023

2. REPORT TYPE
Annual

3. DATES COVERED
01Jun2022 - 30May2023

4. TITLE AND SUBTITLE

Validating Novel Brain Imaging Biomarkers for Classifying Mild Traumatic Brain Injury and Subsequent Risks of Alzheimer's Disease in Gulf War Veterans

5a. CONTRACT NUMBER
W81XWH-20-1-0236

5b. GRANT NUMBER
50208167

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Trustees of Boston University
72 E Newton St M 921
Boston MA 02118-2340

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The central objective in this project is to investigate the utility of the novel MRI markers in determining progressive neurological damage in veterans with mild traumatic brain injury (mTBI), estimate the probability of AD progression based on the neuroimaging proximity measures between mTBI and AD prognostic imaging markers, and build a computational model to provide accurate classification of mTBI and prediction of subsequent risk of AD. In the third project year, we processed and analyzed the second time point MRI scans from the veterans. Machine learning classifier for AD risk has been continuously updated and tested. We also added a normative brain modeling to analyze data in a large-scale point of view. However, the second time point data acquisition is still on-going, and thereby, one year no-cost extension (NCE) was submitted and got approved in this project year.

15. SUBJECT TERMS
Gulf War Illness, Mild Traumatic Brain Injury, AD, Neuroimaging, Immune markers, Cognitive markers, Machine learning

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRDC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	13	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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Introduction

Traumatic brain injury (TBI) is defined as an injury sustained from external forces to the head which eventually lead to alteration or loss of consciousness (LOC). Mild TBI (mTBI) is the most common traumatic brain injury affecting military personnel. Previous reports showed that more than 15 percent of returning service members experienced mTBI and the rate is even higher (~30%) in the large, longitudinally followed Ft. Devens cohort and the Boston Gulf War Illness Consortium (GWIC) of Gulf War (GW) veterans [Hoge et al., 2008; Yee et al., 2016]. In fact, a recent survey found that even mTBI without LOC was associated with more than 2-fold increase in the risk of dementia in veteran populations in their later life compared to those who did not have history of mTBI [Yee et al., 2017]. This suggests that mTBI might have long-term neurodegenerative consequences. It is also possible that layering a mTBI incident over other GW-related or AD risk factors (e.g., genetic, or other health risks) may increase detrimental effects to the brain and result in AD progression. However, detecting and evaluating ongoing pathology in mTBI has been challenging due to the lack of standardized imaging and analysis methods. This also limits our understandings of commonalities in underlying neuropathobiological progression in mTBI and AD.

The central objective in this project is to investigate the utility of the novel MRI markers in predicting progressive neurological damage in veterans with mTBI, estimate the probability of AD progression based on the neuroimaging proximity measures between mTBI and AD prognostic imaging markers, and build a computational model to provide accurate classification of mTBI and prediction of subsequent risk of AD. In this project year, we have been continuously processing new second time point MRI scans transferred from GW longitudinal MRI and Boston Brain Biorepository, Recruitment & Integrated Network for GWI project (Dr. Sullivan in PI of these GW studies). To examine the association between mTBI without LOC and AD risk, some new imaging features are also extracted from Alzheimer's Disease Neuroimaging Initiative (ADNI) data collection. Based on the collected markers, PI and his team have been analyzing the longitudinal change patterns in the veteran data and testing complex computation frameworks to provide time and cost-efficient data to answer the question of who is likely to develop AD after mTBI.

Keywords

Mild TBI
Gulf War Illness
White matter integrity
Gray matter microstructure
Connectivity
Morphometry
Neuroinflammation
MRI marker
Machine Learning
Brain mapping

Accomplishments

- **What were the major goals of the project?**

Major tasks 6 and 7 are relevant to this project year and summarized below. The primary goals of each task are highlighted in the 'Milestone(s) Achieved' cells in the table below.

Specific Aim 1: Identify the impact of microstructural damage responses to mTBI in 3~8 years follow up data on neurological, cognitive and symptom profiles.	**Timeline**	**Site 1**	**Outcomes and Reactions**
Major Task 6 : Analysis on 2nd time point data - final	Months		
Subtask 1: Morhometry and diffusion data processing on rest of 2nd time point dataset (additional 40 subjects processing)	23~28	Dr. Koo, Dr.Sullivan – managing data transfer from GWIC/BBRAIN	As of May 2023, total 69 subject's 2nd time point data was transferred from GW longitudinal study and processed in PI's laboratory. *Work will be continued to the NCE period.*
Subtask 2: Repeat Major task 3 with complete 2nd time point dataset.	28~30	Dr.Koo	Preliminary Analysis on part of data. *Work will be continued to the NCE period.*
Milestone(s) Achieved: same as task 3 with completed dataset			
Major Task 7 : Build Classifier – mTBI classification based on features from Aim 1	Months		
Subtask 1: Build Classifier – mTBI classification based on features from Aim 1	30~33	Dr.Koo	*Work will be continued to the NCE period.*
Subtask 2: Build Classifier – AD classification based on features from Aim 2	30~33	Dr. Koo and Sullivan	ADNI classifier was tested. New computational tool has added to the project. *Work will be continued to the NCE period.*
Milestone(s) Achieved: 1. classifiers 2. descriptions of important features and their relationships	30~33		
Major Task 7 : Build Classifier – nonimaging markers to predict quartile grouping (defined in Aim 3)	Months		
Subtask 1: Build Classifier	30~33	Dr.Koo Dr.Sullivan – managing data transfer from GWIC/BBRAIN	*Work will be continued to the NCE period.*
Milestone(s) Achieved: 1. classifiers 2. descriptions of important features and their relationships			

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

1) Major activities:

Major activities in this project year include followings:

- Processing of 2nd time point data using the pipelines built in the previous year: Newly added time point 2 data in Boston Biorepository, Recruitment & Integrated Network for GWI (BBRAIN) was transferred to the secured parallel computing server located in Boston University's scientific computing cluster network and processed using the multimodal neuroimage processing pipeline built in year 1 (see Table 1 below).

	Baseline (GWIC)		Follow-up (BBRAIN)	
MRI data	Data collected	Data processed	Data collected	Data processed
T1 structural	167	100%	69	100%
T2-TSE	163	0%	69	0%
2D/3D pCASL	151	0%	69	0%
Diffusion MRI	145	100%	69	81%
rs-Functional MRI	157	100%	67	100%

Table 1. Baseline and 2nd time point data processing report.

The processing includes,

- Cortical surface modeling and defining regional cortical structures
- Co-registration between structural and diffusion MRI
- Diffusion data preprocessing for correcting motion and eddy current distortions
- Diffusion modeling on following diffusion indices NODDI and generalized q-space imaging (GQI) maps.
- Structural connectivity matrix reconstructions and network measures (based on graph-theory).
- Cortical diffusivity mapping
- Diffusion sampling along WM major fiber pathway
- Global Morphometry (i.e., volume, gyrification, etc.)
- Regional Morphometry (i.e., regional volume, thickness, etc.)
- Myelination mapping
- Cortical intensity profile mapping
- Hippocampal subfield volume
- Quality assurance work on the processed results. We performed repeated quality assurance works (i.e., detailed visual inspection, checking modeling errors, distributions, and outliers in the quantification values) on the processed results.

- Also, in year 3, we added some new image processing steps as follows,
  --Ravens map (deformation field map to template): This feature was added for brain age estimation based on 2 channel deep learning model (ref).
  --Lobular morphometric measures: This feature was added for testing normative brain modeling (ref).

- Longitudinal data analysis: Although the second time point data acquisition work is still on-going, we performed preliminary data analysis using existing two time point scans. We have assessed longitudinal change patterns of each imaging markers to fingerprint mTBI relevant feature patterns. We also added a new analysis model to derive multivariate pattern proximity measures based on normative aging profile built from healthy control population data. This model provides a measure on how veterans' brain profile is distant to normative brain aging profile.

- Analyzing GWIC data using ADNI: Newly added image processing steps in year 3 were also applied to ADNI MRI data. Imaging markers collected from ADNI scans were used to two different computational modeling approaches to assess AD relevant patterns in GW veterans' data as follows:

--Multivariate pattern proximity assessments between GWIC and ADNI

--Building and testing ML models classifying AD: We are currently testing deep learning classification model in addition to machine learning models reported in the previous year. We will update the results in the final report.

2) Specific objectives:
In the third project year, the main goal was to process all second time point data and complete the planned data analyses as well as building computational models.

3) Significant results and Key outcomes:

- Longitudinal pattern analysis (preliminary):

-- Recapitulation on mTBI imaging markers: we previously reported following patters that may be relevant to mTBI in GWIC data.

a) Cortical microstructural measures (neurite density, a.k.a. ND, or micro-diffusivity, a.k.a. mD) in bilateral anterior cingulate cortex are sensitive to draw differences between mTBI and noTBI in GWIC data. In the white matter, anterior callosal tract diffusivity measures showed significant difference between mTBI and noTBI.

b) Morphometrical analysis, intensity network profile in the GM and white matter (WM) tissue boundary (GWMR) was also significantly different in anterior cingulate region in case_TBI+ vs. controls (figure 1B, left), which overlaps to the findings from diffusion MRI

c) Anterior cingulate diffusion measures were associated with glial activation captured by Translocator protein Positron Emission Tomography (ref)

-- Longitudinal change in microstructural diffusion markers in anterior cingulate cortex: We have observed increased micro-structural orientation dispersion (OD) in mTBI, while noTBI group revealed no change in the same measure. OD could be considered as a minor change secondary to changes in ND. This

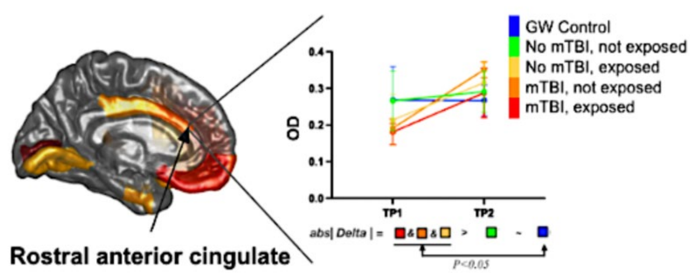

Figure 1. longitudinal analysis of cortical diffusivity

pattern may be an indicative of slow but on-going tissue microstructural damage and resultant neuroinflammatory response in the same region (Cheng et al., 2020). Note that, these findings are still preliminary, and we will need to replicate the analysis once the follow up data acquisition is done in no-cost extension (NCE) period. We expect to have clearer findings from the follow up work.

a) We also found that other war-related risk exposure factors (self-report information , e.g., pesticide, scud missile etc.) could be also important contributors to the on-going cortical microstructural changes in anterior cingulate cortex, when those factors are reported together with mTBI.

b) Further, longitudinal analysis on gray/white matter intensity ratio (GWR), which is known to decrease with aging (Salat et al., 2009), showed significant decreases (P=0.03, T=-2.44) in the rostral cingulate cortex in exposed GWI veterans with mTBI compared to their non-exposed, non-mTBI counterparts.

-- Longitudinal change in the holistic brain measure: We applied a normative brain aging model (Bethlehem, 2022) to assess how veterans' brain profile deviates from normal aging pattern and assess whether the follow-up data showed higher deviations then the baseline indicating on-going abnormal brain aging process. In the baseline data, mTBI showed higher distance measure (i.e., higher deviation from normal aging profile) than noTBI group (avg difference=0.8, arbitrary unit). Interestingly, in the follow-up data, we confirmed more difference between two groups in their distance measure (avg difference=2.9), which was more than 3 times higher than noTBI. This may indicate that neurological change patterns in mTBI are happening more drastically and globally then noTBI.

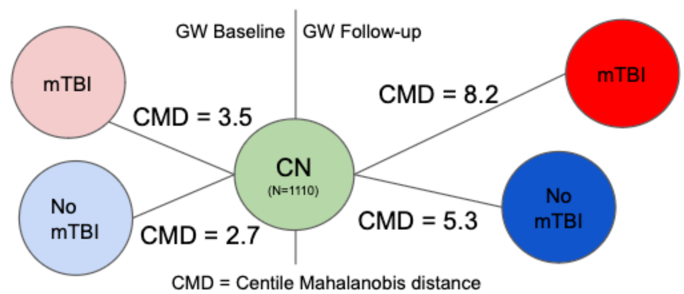

Figure 2. normative brain model analysis.

-- GWIC baseline vs. ADNI: we investigate the aging profiles of GWV (N=166, 142 GWI, 134 males, 53±6.2 yrs.) in comparison to AD subjects (N=318, 179 males, 75.64±6.9), and cognitively normal (CN) subjects (N=1110, 510 males, 65.36±14.8). Based on gray matter volume (GMV) segmented with FreeSurfer (v6), we observed overall significant GMV reduction in GWV compared to the CN group, with more severe reduction seen in GWI veterans with mTBI and exposures (Fig. 3, light to dark blue cells in the matrix). Morphologically, GWI veterans with mTBI and exposures resembled the AD group in lower frontal

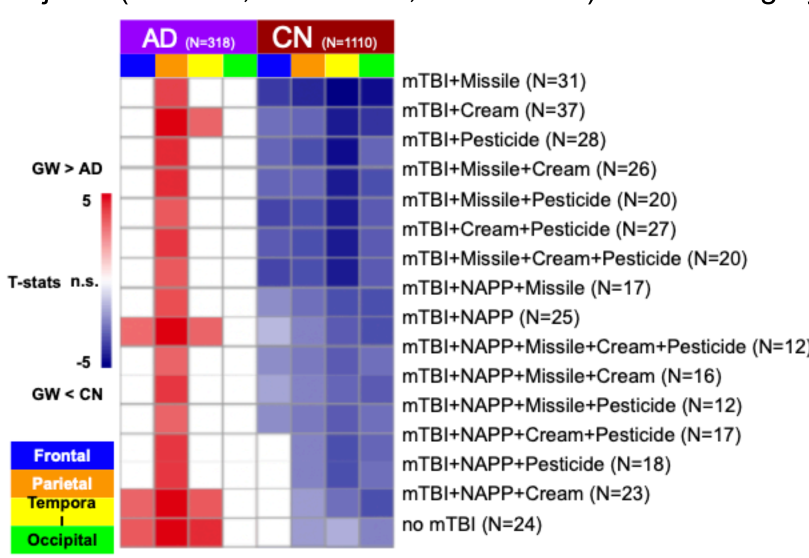

Figure 3. GWIC vs. ADNI multivariate proximity assessments.

and temporal volumes (Fig 3. white cells in the matrix). Interestingly, veterans without mTBI exposure or veterans with mTBI but not exposed to either Missile, Cream, or Pesticide revealed relatively intact morphological profiles in frontal and temporal cortex (Fig 3., red cells in the matrix) than others. We will expand this analysis once we have a complete set of the second time point data.

4) Other achievements:

a. Deep learning-based brain age prediction model: We have been testing deep learning based brain age prediction model using multi-model aggregation scheme (ref). Average difference between predicted and actual age (i.e., brain age delta) was less then 1 years when we tested on GWIC data. This is a big improvement compared with our previous model reported last year. Final testing is still on-going, and we will list the result in the final report after NCE. Also, predicted brain age in veterans will be used together with AD classification measure to build the final prediction model.

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

PI has been attending group meetings in BBRAIN (PI: Kimberly Sullivan, co-I in this project) to discuss new ideas and findings.

PI's group has been doing weekly project meetings (every Friday morning).

PI's group has been doing weekly collaboration meetings with Dr.Yangming Ou at Boston Children's hospital to discuss current deep learning topics (every Friday afternoon).

PI received BU Marion and Henri Gendron Faculty Award for AD research (Jan 2023).

- **How were the results disseminated to communities of interest?**

Nothing to report.

- **What do you plan to do during the next reporting period to accomplish the goals?**

For the next reporting period, we will continuously work on processing the 2nd time point GW dataset and finalize building the different types of machine learning models to provide efficient computational framework for estimating mTBI and AD risks in GW veterans.

Impact

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

  -Our longitudinal image analyses suggest that microstructural damage in the anterior cingulate cortex is a key region for understanding the impact of mTBI in GW veteran's brain. The results also support the view of layering effect, which veterans with either pesticide or harmful chemical exposure can face with severer impact when they also have exposure to mTBI.

  -The normative brain model analysis on longitudinal data provided an important view on that the veterans exposed to mTBI could have more drastic and ongoing impact to their brain than the ones without mTBI. This also suggest that mTBI could be a key contributor to distort the direction of brain aging trajectory impacting their later cognitive aging. Our data comparing GWIC and ADNI additionally support similar view that GW veterans with both mTBI and other exposures (i.e., pesticides and scud missile) might have higher risk of AD than others who did not exposed to mTBI. However, to make clearer conclusion, we still need to add more samples to the analysis. In the next project period, we will continue to process the second time point data and perform additional analyses.

- **What was the impact on other disciplines?**

  Nothing to report.

- **What was the impact on technology transfer?**

  Nothing to report.

- **What was the impact on society beyond science and technology?**

  Nothing to report.

Changes/Problems

- **Changes in approach and reasons for change**

  Nothing to report.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

  PI has been doing discussions with BBRAIN for searching additional imaging data with mTBI information. There was one site (UCSF) from BBRAIN that has both imaging and mTBI classification data. We currently have access to the data and discussing with our colleagues on how to utilize those data to improve statistical power.

- **Changes that had a significant impact on expenditures**

  Nothing to report.

Product

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

Nothing to report

- **Journal publications**

Nothing to report

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

Nothing to report

- **Other publications, conference papers, and presentations.**

2023.04.26 Spring 2023 Colloquium, Umass Lowell, invited talk, "*Deciphering Aging Brain: Imaging Markers of Aging & Data Informatics*". Some of the GWI results were shared in this lecture.

Participants & Other Collaborating Organizations

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

Name: *Bang-Bon Koo*
Project Role: *Principal Investigator*

Name: *Kimberley Sullivan*
Project Role: *co-Investigator*

Name: *Rhoda Au*
Project Role: *unpaid consultant*

Name: *Wendy Qiu*
Project Role: *unpaid consultant*

Name: *Michael Alosco*
Project Role: *unpaid consultant*

Name: *Jasmine Cheng*
Project Role: *research technician*
Nearest person month worked: *7*
Contribution to Project: *Support on developing the computational resources.*

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

Identification of objective brain imaging biomarkers for Gulf War Illness (GWI): exposure- & symptom-specific markers, GW subtypes, and longitudinal patterns
9/01/22-8/31/25

Cal mos.: 2.2
Role: PI

Confirmation of the Low Glutamate Diet as a Treatment for Gulf War Illness
09/01/2022 - 08/31/2025
Cal mos.: 0.6
Role: co-I

Are Tau Proteins in Blood and PET Images Related to Gulf War Illness and Risk of Comorbid Neurological Disorders?
9/01/22-8/31/25
Cal mos.: 1.2
Role: co-I

Installation of a novel *in vivo* BBB imaging protocol to study complex brain aging patterns of AD.
1/1/23-12/31/23
Cal mos.: 1.6
Role: PI

- **What other organizations were involved as partners?**

Nothing to report

Special Reporting Requirements

- **COLLABORATIVE AWARDS:**

Nothing to report

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

Nothing to report

Appendices

Bethlehem, R.A.I., Seidlitz, J., White, S.R. *et al.* Brain charts for the human lifespan. *Nature* **604**, 525–533 (2022). https://doi.org/10.1038/s41586-022-04554-y

Cheng CH, Koo BB, Calderazzo S, Quinn E, Aenlle K, Steele L, Klimas N, Krengel M, Janulewicz P, Toomey R, Michalovicz LT, Kelly KA, Heeren T, Little D, O'Callaghan JP, Sullivan K. Alterations in high-order diffusion imaging in veterans with Gulf War Illness is associated with chemical weapons exposure and mild traumatic brain injury. Brain Behav Immun. 2020 Oct; 89:281-290. PMID: 32745586; DOI: 10.1016/j.bbi.2020.07.006;

Hoge CW, Castro CA, Messer SC, McGurk D, Cotting DI, and Koffman RL. (2008) Combat duty in Iraq and Afghanistan, mental health problems and barriers to care. *US Army Med Dep J.* Jul-Sep:7-17.

Salat D., Lee S., van der Kouwe A., Greve D., Fischl B., Rosas H. (2009). Age-associated alterations in cortical gray and white matter signal intensity and gray to white matter contrast. *Neuroimage* 48 21–28. 10.1016/j.neuroimage.2009.06.074

Yee MK, Seichepine DR, Janulewicz PA, Sullivan KA, Proctor SP, and Krengel MH. (2016) Self-Reported Traumatic Brain Injury, Health and Rate of Chronic Multisymptom Illness in Veterans From the 1990-1991 Gulf War. *J Head Trauma Rehabil.* Sep-Oct;31(5):320-8.

Yee MK, Janulewicz PA, Seichepine DR, Sullivan KA, Proctor SP, Krengel MH. (2017) Multiple Mild Traumatic Brain Injuries Are Associated with Increased Rates of Health Symptoms and Gulf War Illness in a Cohort of 1990-1991 Gulf War Veterans. *Brain Sci.* Jul 9;7(7). pii: E79.