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TITLE: Identification of Epigenetic Signatures as Biomarkers of Gulf War Illness

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CONTRACTING ORGANIZATION: Naval Research Laboratory, Washington, DC

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Introduction

The objective of this research proposal is to use integrated approaches to investigate epigenetics changes of GWI and correlate the epigenetic changes with GW-relevant exposures (PB, pesticides, sarin) to identify the biological indicators in GWI pathogenesis in a well-established cohort of Gulf War veterans from the large multi-site DOD-funded Boston Gulf War Illness Consortium (GWIC) (GW120037). Identified epigenetic changes associated with GWI could potentially provide additional treatment options for veterans with GWI by targeting these changes. It is the aim of this study to identify epigenetic risk factors for GWI by combining the extensive data collection efforts of the Boston GWIC with the epigenetics expertise of the Naval Research Laboratory investigators.

To achieve the objective of this proposed research, the following specific aims will need to be addressed.

Specific Aim 1: Identify DNA Methylation patterns specific to GWI

Specific Aim 2: Discover the changes in microRNA profiles associated with GWI

Specific Aim 3: Apply high performance computing (HPC) bioinformatics approaches to characterize GWI pathogenesis from multiple analytical modalities

Keywords: DNA Methylation, microRNAs, Gulf War Illness, bioinformatics

Accomplishments

This research uses integrated approaches to investigate epigenetics changes of GWI and correlate the epigenetic changes with GW-relevant exposures (PB, pesticides, sarin) to identify the biological indicators in GWI pathogenesis in a well-established cohort of Gulf War veterans from the large multi-site DOD-funded Boston Gulf War Illness Consortium (GWIC) (GW120037). The most significant accomplishments in the initial year was developing protocols and obtaining their approval of human subject research plans so that samples could be received. A meeting with contributors from the University of Boston occurred to finalize experimental plans. Supplies and equipment were ordered to facilitate experimental plan. A more detailed outline of statement of work tasks and accomplishments related to each follows.

Major Task 1: Collect blood samples from archive study participants

The samples were identified from the Boston GWI consortium biorepository but final transfer was not executed in FY 18 due to the lateness of arrival of funds. Arrangements for 60 samples to be shipped were completed in FY 19. After communication the managers of the consortium biorepository it was identified that they were still in the process of collecting samples for the repository. Ongoing delays have also occurred do to miscommunication on details of contracts. These have largely been resolved and arrangements for the final samples should be completed by the middle of FY 19. These arrangements resulted in plans to ship final samples in FY20. They did not actually arrive until Nov. 2020.

Major Task 2: Determine DNA Methylation patterns

The first task that has been completed under this task in the second year of the project was development of the protocol to process the samples. The protocol was tested using none study samples to ensure effectiveness and has been applied to the samples that are on hand. A sequencing facility to have the samples processed at was identified and a contract was set in place. The initial samples that arrived in FY 19 have been processed and results are on hand. A contract has been put in place to allow the remaining samples to be processed which were not received until Nov. 2020. Further samples will be processed and sent for sequencing in batches as they are received.

Major Task 3: Examine microRNA profiles

The majority of the subtasks for this task are also goals for the second and third year of the project. Protocol development is also complete for this task. The samples on hand have been

processed and sequenced for the microRNAs using a microarray technology that was identified in the FY 18. Supplies were purchased to process the remaining samples which arrived on Nov 2020.

Major Task 4&5: Integrated bioinformatics analysis & Reporting

These tasks require large portions of task 2 and 3 to be completed before starting due to the final samples not arriving until Nov 2020 none of this task was accomplished during the eriod of performance.

There is nothing to report regarding opportunities for training and professional development. No results were disseminated to communities of interest as none are far enough along to warrant it.

Impact

Nothing to Report.

Changes/Problems

The project overall was plagued with ordering issues. The initial issues were obtaining the samples from the biorepository. There were several miscommunications about requirements to place the order to transfer samples resulting in significant delays. Further communication with the biorepository has also identified that they do not have all the samples required for this study collected yet and are still in the process of collecting them. This misunderstanding has resulted in the final samples not arriving until the period of performance was complete. They in fact arrived in Nov. of 2020.

The Naval Research laboratory has been required to transition its finical system of record in FY 20. There was a 3.5 month black out period when no ordering was allowed. The new system caused confusion in executing payments and the sequencing center that was processing the samples would not accept a contract for the final samples until payment was received for the initial order. This caused delays in executing tasks. Finally the arrival of COVID and restrictions associated with it in Feb of 2020 did not place significant challenges to our executing tasks at the lab but did further slow down communication with others. This resulted in delays that ultimately prevented the samples from arriving before the end of the period of performance. Due to the fact that the initial award had already been adjusted by one FY to account for delays in placing funds at NRL although a no cost extension was requested it was not possible to grant. The final processing and writing up of results will occur after the end of the period of performance.

Products, Inventions, Patent Applications, and/or Licenses

Publications

Nothing to Report

Technologies or techniques

Nothing to Report

Inventions, patent applications

Nothing to Report

Participants & Other Collaborating Organizations

Name: Dr. Anthony P. Malanoski

Project Role: Principal Investigator

Researcher Identifier: 0000-0001-6192-888X

Nearest person Month worked:5

Contributions to Project: Coordinated efforts, communicated with collaborators to obtain samples

Name: Dr. Tomasz Leski

Project Role: Research Biologist

Researcher Identifier: 0000-0001-7688-9887

Nearest person Month worked:5

Contributions to Project: Developed experimental protocols, obtained necessary supplies for project.

There have been no significant changes to active sources of support.

Organization: Boston University School of Public Health (BU)

715 Albany Street, T4W

Boston, MA 02118

Site PI: Kimberly Sullivan

Partner's contributions are collaborative in form.

The source of the samples if the biorepository established by Dr. Sullivan's ongoing work.

She provided expertise and did required work on her side to existing IRB protocols to allow for transfer of samples from the biorepository to NRL.

Organization: Nova Southeastern University

3301 College Avenue

Fort Lauderdale, FL 33314

Partner's contributions are collaborative in form and involved the actual selection of samples from biorepository that will be transferred to NRL.

Special Reporting Requirements

Nothing to report

Appendices

No appendices attached to this report.