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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 20375

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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: Obtain human subjects approvals and Collect blood samples from study participants

This task has been largely completed except for the final transfer of samples to NRL for further processing. This task included coordination to ensure samples could be transferred and coordination and determination of IRB approvals at all sites. Dr. Kimberly Sullivan made all necessary modifications to her existing human subject protocols to encompass the work of this study. Dr. Anthony Malanoski worked with his local IRB board and a determination was made that the portion of study at NRL would have exempt status as long as the samples transferred were appropriately anonymized. 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 which did not allow sufficient time for sample collection and execution of required contracts. It is anticipated that this will be completed in the beginning of FY19. This will likely result in completion of all subtasks in task 1 within 12 months of receipt of funds although not in the first Fiscal Year and current progress could be estimated at 90%.

Major Task 2: Determine DNA Methylation patterns

The majority of the subtasks for this task are to be goals for the second and third year of the project and so none of the tasks are complete and many are not started as the samples must be transferred before they can be started. The one task that began under this task in the first year of the project was development of the protocol to process the samples. This task was started and is roughly 25% complete with information transferred between groups on how samples are stored so that test extractions of test samples stored in the same manner as biorepository samples can be used to optimize the protocols.

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 and so none of the tasks are complete and many are not started as the samples must be transferred before they can be started. Protocol development is also proceeding for this task as that is the one goal the began in the first year of the project. A new technology to sequence the microRNAs was identified and is being pursued to ensure rapid completion of this task.

Major Task 4&5: Integrated bioinformatics analysis & Reporting

These tasks require large portions of task 2 and 3 to be completed before starting and will not begin until the third year of the project so no progress has been made on this task in the first year of the project.

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.

In the next year of the project that samples will have been transferred and work will proceed on processing samples and submitting to high throughput sequencing. It is anticipated that this portion of the work will be largely completed for in the upcoming year. As results of sequencing are received the analysis and bioinformatics processing of results will also begin and while not completed in the second year the scope of what analyses to be performed will be outlined.

Impact

Nothing to Report.

Changes/Problems

The most significant difficulty was the delay in receipt of funds until mid january which required adjustments to outgoing contracts and resulted in it not being possible to execute a contract to allow for the transfer of samples in the first fiscal year. The fact that the expiration date of the funds was firmly set at Sept 30 without possibility of extension but the funds only being received in January makes it difficult to ensure that contracts will be completed by the expiration date of the funds which is a requirement of the Naval Research Laboratory so the contract for the transfer of samples could not be executed in FY18.

The other change that occurred was that one of the intended participants at NRL could not work on the project. For the tasks for the first year of the project the effort of Tomas Leski, another participant, was increased to allow tasks for the first year to complete in a timely fashion. Ongoing efforts at NRL are proceeding to find a substitute participant to keep tasks on schedule in the upcoming year.

No other significant changes of problems were encountered in the first year of the project.

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.