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TITLE: Investigating Gene-Environment Interactions in Multiple Cohorts of 1990-1991 Gulf War Veterans

PRINCIPAL INVESTIGATOR: Patricia Janulewicz Lloyd, DSc, MPH

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None

14. ABSTRACT

While the 1990-91 Gulf War was relatively short, military personnel who served in the war have suffered long-lasting health consequences. The constellation of health symptoms known as Gulf War Illness (GWI) affects up to one third of 1991 Gulf War veterans. Epidemiologic studies have consistently identified neurotoxicant exposures as the most prominent risk factors for GWI, but an important question remains concerning why some veterans became ill after the war, while others with similar exposures did not. The most prominent exposures of concern include: 1) prolonged use of pyridostigmine bromide (PB) pills, a carbamate compound, 2) excessive use of pesticides (including organophosphates (OPs) and carbamates) and 3) chemical nerve agents (OPs). These compounds have a shared mechanism of action in that they inhibit acetylcholinesterase (AChE) and alter levels of the neurotransmitter acetylcholine, with potential acute and long-term effects on the brain and nervous system. Genetic variability in veterans' ability to neutralize these compounds has long been hypothesized to explain why some veterans got sick and others remained healthy. The human body produces enzymes that protect against adverse effects of cholinergic toxicants, including the enzyme butyrylcholinesterase (BChE). Recent findings from Steele et al (2015) provided preliminary evidence that veterans with slow-acting BChE genetic variants may have been at dramatically increased risk for GWI (OR = 40.0, p = 0.0005) if they used PB during deployment. An additional enzyme, paraoxonase-1 (PON1), catalyzes the hydrolysis of OPs and has also been suggested as a factor that contributed to differences in vulnerability to Gulf War neurotoxicants in theater. The proposed study will build on these preliminary findings and utilize newly collected and existing data from 4 independent cohorts to provide a detailed evaluation of the

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1. INTRODUCTION:

Subject: The subject of this study is to determine the BChE genotype and phenotype in numerous Gulf War veterans who were involved in multiple cohort studies.

Purpose: The purpose of this study is to examine the interaction between genes (BChE and PON1) and environment (exposures in theater) in multiple cohorts of 1990-91 Gulf War veterans.

Scope: Biological samples from X Gulf War veterans will be analyzed from multiple current and prior studies using stored samples.

2. KEYWORDS:

Gulf War, Veterans, Gulf War Illness, Exposure, Pesticides, BChE activity, BChE genotype, PON1 status, PON1 genotype,

3. ACCOMPLISHMENTS:

- What were the major goals of this study:

Major Task 1: Obtain IRB approval
Major Task 2: Obtain blood samples from three sites
Major Task 3: Conduct BChE genotypic and phenotypic analyses
Major Task 4: Perform BChE data analyses
Major Task 5: Collect all PON1 data from the four cohorts and merge with BChE and gene-exposure data set.
Major Task 6: Data Analysis for gene-exposure analysis
Major Task 7: Disseminate study results
Major Task 8: Write annual and final reports for DoD
- What was accomplished under these goals: The major goals accomplished during the past year of the study include obtaining local IRB approval and HRPO approval, shipping and receiving biological samples at the University of Washington and the commencement of analyzing those biological samples. The modified statement of work with information from the original statement of work and the new column added to address the actual timeline of progress can be found below. Specifically, task 4 through 7 have not yet been addressed in this study. However, task 5 through 7 were not intended to be addressed in year 1. Task 4 was originally designed to be addressed in months 6-28 but has not been modified to be addressed in months 12-28.

Major Task 1: Obtain IRB approval
Local IRB approvals have been obtained from each of the local sites as well as from HRPO
Major Task 2: Obtain blood samples from three sites
We have currently obtained, shipped and received biological samples from 469 GW veterans and these samples are now located at the University of Washington.
Major Task 3: Conduct BChE genotypic and phenotypic analyses
Samples from 262 veterans have been analyzed and PON1 activity from these subjects has been determined. Samples from these same 262 veterans have been analyzed and BChE has been examined using the Ellman assay.
Major Task 8: Write annual and final reports for DoD
The PI has completed the annual report for the first year of the study.

Research Specific Tasks:	Timeline (Initial)	Timeline (Actual)
Major Task 1: Obtain IRB approval	months	Months
Subtask 1: Obtain local IRB approvals or exempt status	1-3	1-3
Subtask 2: Obtain HRPO approval or exempt status	3-6	1-6
Milestone(s) Achieved: Obtained local IRB and HRPO approvals	1-6	
Major Task 2: Obtain blood samples from three sites		
Subtask 1: Send first batch of serum samples from sites 1, 4 and 5 (N=433) to the University of Washington for analysis.	6-12	6/12
Subtask 2: Send second batch of serum samples from sites 1, 4, 5 (N=217) to the University of Washington for analysis.	12-24	N/A
Milestone(s) Achieved: All biological samples shipped to the University of Washington for analysis.	6-24	
Major Task 3: Conduct BChE genotypic and phenotypic analyses		
Subtask 1: Run BChE assays at University of Washington to determine genotype and phenotype for each serum sample from first shipment from sites 1,4 and 5 (N=433)	6-12	6-12
Subtask 2: Run BChE assays at University of Washington to determine genotype and phenotype for each serum sample from second shipment of sites 1, 4 and 5 (N=217)	12-24	N/A
Milestone(s) Achieved: All serum samples (N=650) will have genotypic and phenotypic data for BChE	6-24	
Major Task 4: Perform BChE data analyses		
Subtask 1: Acquire all newly analyzed BChE genotypic and phenotypic results data from University of Washington.	6-28	12-28
Subtask 2: Merge and clean all existing and newly acquired BChE data	24-26	N/A
Subtask 3: Merge clinical dataset data from sites 1, 3, 4, 5 for case/control status, environmental exposures and demographics with results from laboratory analyses.	18-30	N/A
Subtask 4: Perform preliminary analyses	26-30	N/A
Milestone(s) Achieved: Completed dataset with all (N=834) BChE genotypic and phenotypic data and preliminary data analyzed.	6-30	
Major Task 5: Collect all PON1 data from the four cohorts and merge with BChE and gene-exposure data set.		

Subtask 1: Collect data from Dr. Chao's PON1 study of the same four cohorts	20-26	N/A
Subtask 2: Merge PON1 dataset with BChE, environmental exposure and GWI case definition dataset	26-32	N/A
Subtask 3: Perform preliminary analyses	30-32	N/A
Milestone(s) Achieved: Completed dataset with all (N=834) PON1 and BChE, data and preliminary data analyzed	32	
Major Task 6: Data Analysis for gene-exposure analysis		
Subtask 1: Perform gene-exposure (BChExPON1xexposure) data analyses	28-36	N/A
Milestone(s) Achieved: Gene-exposure final data analysis completed	12-36	
Major Task 7: Disseminate study results		
Subtask 1: Present BChE, PON1 and gene-exposure analyses results at scientific conferences	12-32	N/A
Subtask 2: Prepare BChE and PON1 gene-exposure outcome manuscripts and submit for publication	28-36	N/A
Milestone(s) Achieved: preliminary data presented at annual scientific conferences and manuscript submission of final data analysis	28-36	
Major Task 8: Write annual and final reports for DoD		
Subtask 1: Write annual reports for DoD	12, 24	Current
Subtask 2: Write final report for DoD	36	N/A
Milestone(s) Achieved: Annual and final reports will be submitted to DoD	12, 24, 36	

- What opportunities for training and professional development: Nothing to Report

- Were the results disseminated to communities: Nothing to Report

- What do you plan to do during the next reporting period: Over the next reporting period I plan to obtain the remainder of the biological samples available from each of the GW veteran cohorts and ship them to the University of Washington. In addition, I plan to 1) continue to conduct BChE assays on the remaining samples, 2) acquire data from UW on BChE and PON1 activity, 3) start to merge demographic and exposure data from each of the cohorts and 4) conduct preliminary analysis.

4. IMPACT: Nothing to Report yet

5. CHANGES/PROBLEMS: Samples from one of Dr. Chao's cohorts had been stored at the University of Washington and it took longer than anticipated to uncover them in the freezers at UW as over the years there

were multiple freezer location changes. All samples have now been discovered. It was also discovered that not all biological samples had been used with the type of collection tubes necessary to determine BChE genotype. However, it was determined that saliva samples could be used to conduct BChE genotyping and fortunately, saliva samples were available for those veterans. We obtain local IRB approvals at each site to use stored saliva as well as HRPO approval.

6. PRODUCTS: Nothing to Report

7. PARTICPANTS & OTHER COLLABORATING ORGANIZATIONS

- What individuals have worked on the project?

Name:	Patricia Janulewicz Lloyd, DSc, MPH
Project Role:	Principal Investigator
Nearest person month worked:	2.3
Contribution to Project	Dr. Janulewicz Lloyd has been the oversight of the entire project. She has worked with each of the co-Investigators to get biological samples shipped from their locations and has worked on the creation of the database for merged data.

Name:	Kimberly Sullivan, PhD
Project Role:	Co-Investigator
Nearest person month worked:	0.4
Contribution to Project	Dr. Sullivan was involved in getting biological samples shipped from the GWIC biorepository to the University of Washington

Name:	Christine Lloyd Travaglini
Project Role:	Data Analyst
Nearest person month worked:	0.69
Contribution to Project	Ms. Travaglini has worked on creating the structure of the dataset in which all of the demographic variables, exposure information and phenotypic and genotypic data will be merged from all cohorts

- Has there been a change in the active other support of the PD/PI or senior/key personnel since the last reporting: Nothing significant to Report
- What other organizations were involved as partners?

1. Organization Name: University of Washington

Location of Organization: Seattle, Washington

Partner's contribution to the project: Drs. Clement Furlong and Judit Marsillach and Ms Rebecca Richter

Facilities: All phenotypic and genetic analyses for this project are and will be conducted at the University of Washington using their equipment.

Collaboration: Drs. Furlong and Marsillach and Ms. Richter have been collaborating with the PI to obtain all necessary biological samples necessary for the completion of this study.

2. Organization Name: University of California San Francisco

Location of Organization: San Francisco, California

Partner's contribution to the project: Dr. Linda Chao

Collaboration: Dr. Chao has been collaborating with the PI and the collaborators at the University of Washington to locate all necessary biological samples necessary from the two cohorts of veterans that Dr. Chao has worked with and has biological samples from and will be used in this study.

8. SPECIAL REPORTING REQUIREMENTS: Nothing to Report

9. APPENDICES: Nothing to Report