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PRINCIPAL INVESTIGATOR: Linda Chao

RECIPIENT: Northern California Institute for Research and Education (NCIRE), San Francisco, CA

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14. ABSTRACT

The goal of this project is to evaluate the extent to which $PON1_{192}$/Gulf War (GW)-related exposure interactions contribute to the risk for Gulf War Illness (GWI), as defined by the Centers for Disease Control and Prevention (CDC) and Kansas case definitions in a large sample of GW veterans. Specifically, we will: (1) determine the associations between GWI and GW-related exposures with the potential for "cholinergic" effects (e.g., personal pesticide use, exposure to OP nerve agents) in subgroups of veterans with different $PON1_{192}$ genotype. (2) determine the associations between GWI and each GW-related "cholinergic" exposure in subgroups of veterans with different PON1 activity levels and (3) calculate prevalence odds ratios for GWI/exposure associations separately for sugbroups of veterans with different $PON1_{192}$ genotypes and PON1 activity levels.

15. SUBJECT TERMS
Gulf War Illness (GWI), Paraoxonase-1 (PON1), organophosphates (OP), Gulf War-related exposures

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1. INTRODUCTION:

The goal of this project is to evaluate the extent to which $PON1_{192}$/Gulf War (GW)-related exposure interactions contribute to the risk for Gulf War Illness (GWI), as defined by the Centers for Disease Control and Prevention (CDC) and Kansas case definitions in a large sample of GW veterans. Specifically, we will: (1) determine the associations between GWI and GW-related exposures with the potential for "cholinergic" effects (e.g., personal pesticide use, exposure to OP nerve agents) in subgroups of veterans with different $PON1_{192}$ genotype. (2) determine the associations between GWI and each GW-related "cholinergic" exposure in subgroups of veterans with different PON1 activity levels and (3) calculate prevalence odds ratios for GWI/exposure associations separately for subgroups of veterans with different $PON1_{192}$ genotypes and PON1 activity levels.

2. KEYWORDS:

Gulf War Illness (GWI), Paraoxonase-1 (PON1), organophosphates (OP), Gulf War-related exposures

3. ACCOMPLISHMENTS:

What were the major goals of the project?

1. Obtain information about GW-related exposures from participants of the completed DoD-funded GW study for whom we have $PON1_{192}$ genotype and PON1 activity data (by 10^{th} month).
2. Obtain $PON1_{192}$ genotype and PON1 activity levels for veterans participating in the on-going VA Merit GW study at the SF VAMC (by month 17).
3. Obtain $PON1_{192}$ genotype and PON1 activity levels on biosamples from the Gulf War Illness Consortium (GWIC) (by month 27).
4. Obtain $PON1_{192}$ status and PON1 activity levels for veterans participating in the re-evaluation study of the Ft. Devens cohort (by month 15).
5. Analyze PON1 and self-reported GW-exposure data (by month 33).

What was accomplished under these goals?

Major activities and achievements:

A. To date, Dr. Furlong's laboratory has established PON1 status, genotype, and phenotype for **546** samples.
    1. 518 of these samples have CDC CMI case status and PON1 status
    2. 391 of these samples have Kansas GWI case status and PON1 status

B. Dr. Lea Steele has performed preliminary analyses on **342** veterans with PON1 and Kansas GWI case status:
    1. Overall, there are no significant PON1 case/control differences in this preliminary sample. This is consistent with our original expectation that the veterans' risk for developing GWI would not be a function of PON1 genotype or activity, but rather any increased GWI risk potentially associated with PON1-192 Q and R alleles would only be observed in veterans exposed to the types of chemical(s) for which their PON1 genotype would potentially put them at risk.
    2. Multivariable modeling with logistic regression identified only 3 significant risk factors for GWI in our sample: (i) hearing chemical alarms, our questionnaire proxy for possible nerve agent exposure, (ii) using pesticide cream or spray on the skin, and (iii) using PB/NAPP pills, with the strongest associations occurring with exposures of one week or longer. This is consistent with previous reports by Drs. Steele, Sullivan, and others.
    3. GWI risk associated with specific exposures in theater did vary with PON1 genotype: Specifically, extended exposure to personal use skin pesticides was only a significant GWI risk factor for veteran carriers of the Q allele and appeared to be most pronounced in QQ homozygotes (Odd Ratio, OR = 7.26, $p<0.001$). However, personal use skin pesticide was not a significant risk factor for PON1 RR homozygotes (OR = 0.90, ns). The PON1 Q allele is known to be ineffective at hydrolyzing certain OP pesticides, compared to the R allele. This finding is consistent with expected risk associated with PON1 Q and provides preliminary support for Study Hypothesis 1 - carriers of the PON1 Q allele are at increased risk for GWI in connection with pesticide use during deployment.
    4. Hearing chemical alarms sounded for 7 days or longer was only a risk factor for veteran carriers of the R allele (OR for QRs = 2.94, $p=0.05$; OR for RRs = 5.31, $p=0.06$), but was not a risk factor for QQ homozygotes. The PON1 R allele is known to be ineffective at hydrolyzing chemical nerve agents. Our finding in consistent with expected risk associated with PON1 R and provides preliminary support for Study Hypothesis 2 - carriers of the PON1 R allele are at increased risk for GWI in connection with exposure to nerve agents during deployment.
    5. An additional preliminary finding not included in our initial hypotheses is that GWI risk associated with use of pyridostigmine bromide (PB) may also have varied with PON1 genotype. PB is a carbamate AChEi compound, given in theater as a prophylactic measure against adverse effects of nerve agent exposure. While PON1 is not generally considered a factor in carbamate hydrolysis, our preliminary finding is intriguing and deserves further evaluation, particularly in light of earlier identification of PB as a prominent risk factor among carriers of certain butyrylcholinesterase genetic variants (Steele at al, 2015) and recent findings (Bosak et al, 2020) that carbamates may reduce PON1 activity.

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

Nothing to report.

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?

- Dr. Steele's group will continue to analyze the data in more detail, looking at other exposures and optimizing the statistical models.
- We plan to write up the preliminary findings for publication in one of the journals that have special issues dedicated to Gulf War Illness research.
- There handful of subjects from the Boston GWIC cohort who do not have PON1 data. We are working reconciling this situation.
-There are 69 subjects from Dr. Krengel's BVARI cohort who do not have information about Kansas GWI exclusionary conditions; therefore, we are unable to discern Kansas GWI case status for these 69 subjects. Dr. Krengel is looking into the possibility of re-contacting these Veterans so that we can obtain information about whether or not they have any conditions that are considered exclusionary for the Kansas GWI case status.

4. IMPACT:

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

The preliminary findings support our hypothesis and suggest that there are differences in the impact of exposures to deployment-related "cholinergic" exposures on GWI by PON1 genotype.

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.

5. 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

Dr. Maxine Krengel was unable to bring back many of the veterans from her 'Redefining Gulf War Illness Using Longitudinal Health Data: The Fort Devens Cohort' study for blood draw and updated information about their symptoms and current health. Instead, she recruited 69 Gulf War Veterans from the Boston VA for blood draw for PON1 status. Unfortunately, she did not to obtain enough information from these Veterans to ascertain a) Kansas GWI case status, b) Kansas GWI exclusionary conditions, and c) GW exposure questions from the Kansas GW Military History and Health Questionnaire. Because the 69 GW veterans from the BVARI cohort have GW exposure data from the Structured Neurotoxicant Assessment Checklist (SNAC), data from these 69 GW veterans could potentially be used for "preliminary/exploratory" type comparisons. However, the SNAC exposure questions differ substantially from the Kansas exposure questions, and do not include the specific variables that were most informative in our preliminary analyses (e.g. use/duration of NAPP/PB pill use, types/duration of pesticide use, chemical alarms, etc.). Furthermore, we do not have SNAC data for GW veterans from the San Francisco cohorts.

Dr. Krengel and her staff will try to re-contact the 69 GW veterans from the BVARI to obtain the missing information. However, it is unclear how successful they will be in this endeavor.

There are a handful of GW Veterans from the Boston GWIC cohort who do not have PON1 data. We are in the process of tracking down the reason for this.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

There are no significant deviations or changes in approved protocols for the use of human subjects. The current IRB approval dates are:

SF VAMC site: initially approved: 03/30/2016; current expiration date: 12/08/2020
Boston University site: initially approved 6/23/16, current expiration date: 6/22/21
Boston VA site: initially approved 11/14/16, current expiration date: 5/6/21
Baylor College of Medicine site: initially approved: 9/13/16, current expiration date: 4/7/21

The University of Washington IRB determined that the UW site is not "engaged" in human subject research. Therefore, the UW IRB decided that IRB approval for the activities conducted by the UW research team is not required.

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:

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

Journal publications.

Nothing to report.

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

Nothing to report.

Other publications, conference papers, and presentations.

Nothing to report.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

N/A

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Linda Chao
Project Role:	PI
Researcher Identifier:	0000-0002-8593-2434 (eRA Commons: lindachao)
Nearest person month worked:	0.6 calendar months
Contribution to Project:	Dr. Chao has work closely with Drs. Furlong, Sullivan, Steele, Krengel, and Klimas to coordinate all aspects of project, from study procedures, data collection, to data quality control.
Name:	Nancy Klimas
Project Role:	Co-Investigator
Researcher Identifier:	0000-0003-1459-3268
Nearest person month worked:	0.24 calendar months
Contribution to Project:	Dr. Klimas' laboratory houses all the GWIC samples. She will oversee the preparation and sending of the GWIC biorepository to Dr. Furlong's laboratory for PON1 analyses.

Name:	Clement Furlong
Organization Name:	University of Washington
Project Role:	Site PI
Researcher Identifier:	0000-0002-6489-7211 (eRA Commons: furlong)
Nearest person month worked:	0.36 calendar months
Contribution to Project:	Dr. Furlong is directing Ms. Richter in determination of PON1 status of the Gulf War veterans in the study.
Name:	Rebecca Richter
Organization Name:	University of Washington
Project Role:	Research Scientist in Dr. Furlong's laboratory
Researcher Identifier:	N/A
Nearest person month worked:	1.0 calendar months
Contribution to Project:	Ms. Richter has been working with Dr. Furlong to determine the PON1 status of the remaining Gulf War veterans' plasma samples from Boston GWIC and the BVARI cohorts using the three-substrate assay/analysis protocol.
Name:	Kimberly Sullivan
Organization Name:	Boston University
Project Role:	Site PI
Researcher Identifier:	0000-0001-7940-6123
Nearest person month worked:	0.36 calendar months
Contribution to Project:	Dr. Sullivan has been providing serum samples and Gulf War-related exposure data from the Department of Defense-funded (GW120037) multi-site Gulf War Illness consortium (GWIC) for study analysis.
Name:	Maxine Krengel
Organization Name:	Boston VA/Boston VA Research Institute (BVARI)
Project Role:	Site PI
Research Identifier:	0000-0001-7632-590X
Nearest person month worked:	0.36 calendar months
Contribution to Project:	Dr. Krengel had been providing serum samples from GW veterans that she recruited from the Boston VA.
Name:	Lea Steele
Organization Name:	Balyor College of Medicine
Project Role:	Site PI
Research Identifier:	0003-4940-069X
Nearest person month worked:	0.6 calendar months
Contribution to Project:	Dr. Steele has worked on adjudicating Kansas GWI case status and preliminary analyses of the PON1 and GW exposure data.

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

Name:	Linda Chao
Changes:	1. VA-funded merit-review grant "An Investigation of the Relationship between Toxicant Exposures during Gulf War Deployment and prodromal Parkinson's disease." Was funded in January 2019. Dr. Chao is PI at 0.7 calendar months. Her effort on this project will increase in January 2021 when her other VA Merit review grant ends. 2. VA-funded merit-review grant "Pilot Test of Telephone-Delivered Cognitive Behavioral Therapy for Insomnia for Veterans with Gulf War Illness" will end in December 31, 2020. Dr. Chao's 11.3 calendar months effort will shift to the other Merit review project. 3. Dr. Chao received a no-cost extension for DOD/CDMRP grant "Using Multimodal Imaging to Examine the Neural Mechanisms of an Integrative Exercise Program for Individuals with Mild Cognitive Impairment." She is PI at 1.2 calendar months. 4. DOD/CDMRP grant "Investigating Gene-Environment Interactions in Multiple Cohorts of 1990-91 Gulf War Veterans" received a no-cost extension. Dr. Chao is co-investigator at 0.18 calendar months. 5. NIH/NIMH grant "Individual Variation in Effects of Traumatic Stress on Gray Matter Myelin" has been funded. Dr. Chao is co-investigator at 1.4 calendar months.
Name:	Kimberly Sullivan
Changes:	1. DOD/CDMRP grant "Investigating Gene-Environment Interactions in Multiple Cohorts of 1990-91 Gulf War Veterans" received a no-cost extension. Dr. Sullivan is co-investigator at 0.6 calendar months. 2. DOD/CDMRP grant "Defining and Characterizing GWI Pathobiology using Longitudinal Brain Imaging Biomarkers of White Matter Integrity and Hemodynamic Response" has been funded. Dr. Sullivan is PI at 0.6 calendar months. 3. DOD/CDMRP grant "Clarifying the Role Played by Microglia and Astrocyte Activation in Veterans with Gulf War Illness Using Positron Emission Tomography (PET)" has been funded. Dr. Sullivan is co-investigator at 0.6 calendar months. 4. DOD/CDMRP grant "Identifying Objective Diagnostic Markers of Gulf War Illness: Salivary and Plasma Autoantibodies Against Neural Proteins Validated With Brain Imaging" has been funded. Dr. Sullivan is co-investigator at 0.6 calendar months. 5. VA-funded merit-review grant "An Investigation of the Relationship between Toxicant Exposures during Gulf War Deployment and prodromal Parkinson's disease." was funded in January 2019. Dr. Sullivan is co-investigator at 0.6 calendar months. 6. Dr. Sullivan's effort on DOD/CDMRP grant "Brain-Immune Interactions as the Basis of Gulf War Illness: Gulf War Illness Consortium (GWIC)" has ended.

Name:	Clement Furlong
Changes:	1. NIH/WE-RESEARCH grant WR-20041-Dx "Highly sensitive and specific immunoresistive sensor for point-of-care screening of COVID-19" has been funded. Dr. Furlong is co-investigator at 0.6 calendar months. 2. National Oceanic and Atmospheric Administration (NOAA) grant "Continued Engineering Improvements to an Embedded Surface Plasmon Resonance Biosensor for Detecting Algal Toxins Using Autonomous Underwater Vehicle Technology" has been funded. Dr. Furlong is PI at 0.36 calendar months.
Name:	Lea Steele
Changes:	1. DOD/CDMRP grant "Investigating Gene-Environment Interactions in Multiple Cohorts of 1990-91 Gulf War Veterans" received a no-cost extension. Dr. Steele is co-investigator at 0.6 calendar months. 2. NIH/NIEHS grant "Gulf Coast Center for Precision Environmental Health (GC-CPEH)" was funded. Dr. Steele is co-investigator at 0.6 calendar months. 3. Dr. Steele's effort on DOD/CDMRP grant "Brain-Immune Interactions as the Basis of Gulf War Illness: Gulf War Illness Consortium (GWIC)" has ended. 4. Dr. Steele's effort on DOD/CDMRP grant "Assessment of Diverse Biological Indicators in Gulf War Illness: Are they Replicable? Are They Related?" has ended.
Name:	Nancy Klimas
Changes:	1. DOD/CDMRP grant "Defining and Characterizing GWI Pathobiology using Longitudinal Brain Imaging Biomarkers of White Matter Integrity and Hemodynamic Response" has been funded. Dr. Klimas is co-Investigator at 0.6 calendar months. 2. VA Merit grant "CMA: Immune/Inflammatory Priming in Exacerbating Responses to GWVI Stressors: Implications for GWVI Treatments" has been funded. Dr. Klimas is co-Investigator at 0.6 calendar months.
Name:	Maxine Krengel
Changes:	1. DOD/CDMRP grant "Gulf War Women's Health Cohort" was funded. Dr. Krengel is co-investigator at 1.2 calendar months. 2. VA-funded merit-review grant "An Investigation of the Relationship between Toxicant Exposures during Gulf War Deployment and prodromal Parkinson's disease." was funded in January 2019. Dr. Krengel is co-investigator at 0.6 calendar months.

What other organizations were involved as partners?
Nothing to Report

8. **SPECIAL REPORTING REQUIREMENTS:**
   N/A

9. **APPENDICES:**
   N/A