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AWARD NUMBER: W81XWH-16-1-0558

TITLE: Examination of plasma PON1 paraoxonase activity and genotype in Gulf War veterans

PRINCIPAL INVESTIGATOR: Linda Chao

**RECIPIENT: Northern California Institute for Research and Education (NCIRE)
San Francisco, CA 94121**

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TYPE OF REPORT: Annual

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

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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 subgroups of veterans with different PON1 ₁₉₂ genotypes and PON1 activity levels.					
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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The goal of this project is to evaluate the extent to which PON1₁₉₂/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 PON1₁₉₂ 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 PON1₁₉₂ genotypes and PON1 activity levels.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

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

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

1. Obtain information about GW-related exposures from participants of the completed DoD-funded GW study for whom we have PON1₁₉₂ genotype and PON1 activity data (by 10th month).
2. Obtain PON1₁₉₂ 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 PON1₁₉₂ genotype and PON1 activity levels on biosamples from the Gulf War Illness Consortium (GWIC) (by month 27).
4. Obtain PON1₁₉₂ 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?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major activities and achievements:

- A. Contacted, consented, and received completed Kansas Military History and Health Questionnaires from **110** participants of previous DoD-funded Gulf War Study for whom we have PON1 information.
- B. Sent blood samples for **128** participants from the PI's now completed VA Merit Gulf War study to Dr. Furlong's laboratory at the University of Washington for PON1 processing and analysis.
- C. Sent blood samples for **114** participants from the GWIC to Dr. Furlong's laboratory at the University of Washington for PON1 processing and analysis.
- D. Sent blood samples for 20 Devens telephone follow-up study to Dr. Furlong's laboratory at the University of Washington for PON1 processing and analysis.
- E. Dr. Furlong's laboratory has established PON1 status, genotype, and phenotype for **372** samples to date.

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

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report.

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report.

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

If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

The Boston VA/BVARI site has an additional **33** samples that they are getting ready to ship to Dr. Furlong's laboratory for PON1 analysis. This site is still recruiting GW veterans from the Fort. Devens cohort for blood samples and to complete the Kansas Gulf War Military History and Health Questionnaire.

The GWIC has an additional **61** samples ready to send to Dr. Furlong's for PON1 analysis. Dr. Sullivan has a no cost extension for the GWIC so this site is also still recruiting GW veterans for blood samples and Kansas Gulf War Military History and Health Questionnaire data.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

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

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report.

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

- 5. CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report.

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

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

No actual or anticipated problems. Because Drs. Sullivan and Kregel are still recruiting GW veterans, we will request a no cost extension to capture as many of these GW veteran samples as possible.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting

Nothing to report.

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

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: 02/23/2017; current expiration date: 2/14/2020

Boston VA/BVARI site: initially approved 11/14/16, current expiration date: 5/6/19

Boston VA site: initially approved 6/23/16, current expiration date: 6/22/19

Baylor College of Medicine site: initially approved: 9/13/16, current expiration date: 6/11/19

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: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

• **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report.

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

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to report.

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

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate

the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *biospecimen collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

N/A

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change.”

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, Kregel, 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.

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

If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Name: Linda Chao

Changes: 1. VA Merit grant 5I01CX000798 has been renewed. New performance period is 01/01/2019-12/31/2023. Effort on this project is 5.28 calendar months.
2. DOD/CDMRP grant “Boston Biorepository, Recruitment, and Integrative Network (BBRAIN) for GWI” (GW170055) was awarded (performance period: 9/1/19-8/31/21). Dr. Chao is Co-Investigator and site-PI. Her effort on this project is 0.24 calendar months.
3. Dr. Chao is co-investigator on NIH grant (R01 MH115020-01A1) “Individual variation in effects of traumatic stress on gray matter myelin” at 0.6 calendar months. The performance period of this project is 09/01/18-08/31/23.
4. Dr. Chao has a joint appointment with the San Francisco VA (87.5% effort) and the University of California San Francisco. Her UCSF appointment has been increased to 26.5%. Dr. Chao’s combined effort may not exceed 115%

Name: Kimberly Sullivan

Changes: 1. DOD/CDMRP grant “Boston Biorepository, Recruitment, and Integrative Network (BBRAIN) for GWI” (GW170055) was awarded (performance period: 9/1/19-8/31/21). Dr. Sullivan’s effort on this project is 2.4 calendar months.
2. DOD/CDMRP grant GW120037 has ended, but is currently on-cost extension. Dr. Sullivan’s effort on this project has been reduced from 3.6 to 2.64 calendar months.
3. DOD/CDMRP grant GW110054 has ended.
4. DOD/CDMRP grant “Novel Autoantibody Serum and Cerebrospinal Fluid Biomarkers in Veterans with Gulf War Illness” has concluded.
5. DOD grant “D-cycloserine –A Novel Treatment for Gulf War Illness” has concluded.
6. Dr. Sullivan’s effort on DOD/CDMRP grant GW150116 has been reduced to 0.6 calendar months.
7. Dr. Sullivan’s effort on DOD/CDMRP grant GW150050P1 has been reduced to 0.6 calendar months.
8. Dr. Sullivan’s effort on “A Randomized, Double-blind Placebo-controlled Phase III Trial of Coenzyme Q10 in Gulf War Illness” grant from the Department of Veterans Affairs has been reduced to 2.04 calendar months.
9. Dr. Sullivan’s effort on DOD/CDMRP grant GW160032 has been reduced to 0.48 calendar months.

10. Dr. Sullivan's effort on DOD/CDMRP grant GW160053 has been reduced to 0.6 calendar months.

11. Dr. Sullivan is co-investigator on VA Merit grant 5I01CX000798 (performance period is 01/01/2019-12/31/2023). Her effort on this project is 0.6 calendar months.

Name: Clement Furlong

Changes: 1. Grant "Role of BPHL/homocysteine thiolactonase in hyperhomocysternia and disease" ended 7/31/17.
2. UW Center for Process Analysis and Control grant "Advance Surface Plasmon Resonance Sensors" was awarded (performance period: 10/1/15-9/30/19). Dr. Furlong is PI contributing 0.6 calendar months to the project.
3. Dr. Furlong is co-PI on NIH/NIEHS grant "Superfund Hazardous Substances Basic Research Program: Effects-related biomarkers of neurotoxic exposures; Project 3: Role of paraxonases in modulating cadmium, manganese, and organophosphate neurotoxicity. (performance period 04/01/18-03/31/20) contributing 1.2 calendar months to the project.

Name: Lea Steele

1. DOD/CDMRP grant GW160077 was awarded (performance dates: 09/30/17-09/29/20). Dr. Steele is a co-investigator contributing 0.3 calendar months to this project.
2. NIH grant Gulf Coast Center for Precision Environmental Health (GC-CPEH) was awarded (performance period is 04/2019 – 04/2024). Dr. Steele is a co-investigator contributing 0.6 calendar months to this project

Name: Nancy Klimas

Change: 1. Dr. Klimas' contribution to NIH grant R01NS090200 has been reduced from 0.972 to 0.9 calendar months. The grant also has a new end date of 7/31/19.
2. Dr. Klimas' contribution to DOD grant W81XWH-13-2-0085 has been reduced from 0.792 to 0.72 calendar months.
3. Dr. Klimas' effort on VA Merit grant "A Translational Medicine Approach to Gulf War Illness: From Cells to Therapy" has been reduced from 1.1 to 0.432 calendar months.
4. DOD grant W81XWH-15-1-0537 has been extended. The new end date is 6/30/19 and Dr. Klimas' effort on this project remains 0.6 calendar months.
5. DOD grant W81XWH-15-1-0163 ended on 12/31/18.
6. NIH grant 1R21AI12487 ended on 3/31/18/
7. NIH grant R15 NS087604-01A is on a no cost extension. The new end date is 9/31/19. Dr. Klimas' contribution has been changed to 0.12 calendar months to this project.
8. Dr. Klimas is PI on DODGWIRP grant W81XWH1820062 (performance period 9/30/18-9/29/22). She is contributing 1.44 calendar months to this project.
9. Dr. Klimas is PI on DOD/GWIRP grant W81XWH-17-1-0683 (performance period 09/01/17-04/30/20). She is contributing 0.36 calendar months to the project.
10. Dr. Klimas is co-investigator on DOD/GWIRP grant W81XWH-17-2-0063 (performance period 10/01/17-09/30/20). She is contributing 0.36 calendar months to this project.
11. Dr. Klimas is co-investigator on DOD/GWIRP grant W81XWH-17-1-0640 (performance period 09/01/17-08/30/19) at 0.2 calendar months.

12. Dr. Klimas is co-investigator on DOD/GWIRP grant W81XWH-18-1-0549 (performance period 09/30/18-09/29/22). She is contributing 0.24 calendar months to the project.

13. Dr. Klimas is co-PI on NIH grant 1R21NS106212-01 (performance period 12/1/18-11/30/20). She is contributing 0.6 calendar months to the project.

Name: Maxine Krengel

Changes:

1. Dr. Krengel is co-investigator and site PI for DOD/CDMRP grant “Gulf War Women’s Health Cohort” (performance period 10/1/17-09/30/20). She is contributing 1.2 calendar months to the project.
2. Dr. Krengel is co-investigator and site-PI on VA Merit grant 5I01CX000798 (performance period is 01/01/2019-12/31/2023). Her effort on this project is 1.2 calendar months.
3. Dr. Krengel is co-investigator on a clinical pilot study called “Transcranial Photobiomodulation to Improve Cognition in TBI with pre-/post-fMRI scans: A pilot, sham-controlled study, funded by Vielight Inc. Her effort on this project is 1.2 calendar months.
4. VA CSR&D funded study (CX000524-01A1) Transcranial, Light-Emitting Diode (LED) Therapy to Improve cognition in GWVI ended on 09/30/18/

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner’s contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner’s facilities for project activities);*
- *Collaboration (e.g., partner’s staff work with project staff on the project);*

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: 0.6 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 Washintgon
Project Role: Research Scientist in Dr. Furlong's laboratory
Researcher Identifier: N/A
Nearest person month worked: 3.0 calendar months
Contribution to Project: Ms. Richter has been working with Dr. Furlong to determine the PON1 status of the 103 Gulf War veterans' plasma samples from Dr. Chao's VA Merit award 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 is 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. Once we receive HRPO clearance for Dr. Klimas' site, which houses all the GWIC samples, 100 GWIC samples will be sent to Dr. Furlong's laboratory.

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: 0.60 calendar months
Contribution to Project: Dr. Krengel is providing serum samples and Gulf War-related exposure data from her call-back survey study of the Fort Devens cohort. She will send 20 samples this month.

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 with study collaborators on study planning and design.

8. SPECIAL REPORTING REQUIREMENTS: N/A

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

N/A