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TITLE: Diagnosis of Late-Stage, Early-Onset, Small-Fiber Polyneuropathy

PRINCIPAL INVESTIGATOR: Jorge M. Serrador, PhD

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Fort Detrick, Maryland 21702-5012

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Approved for Public Release
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The goal of this study is to determine the prevelence of longstanding early-onset small-fiber polyneuropathy (SFPN) in Veterans with Gulf War Illness and to translate this information clinically by developing and validating tools to diagnose SFPN more quickly and reliably. Study participants will undergo several tests to determine occurrence of SFPN, specifically Autonomic Function Testing and skin biopsy to detect morphology and count of nerve endings. These tests will be compared to a symptom evaluation questionnaire for SFPN, a neurological exam that measures muscle strength and the ability to feel different sensations, genomic markers specific to SFPN, and pupil measurements including diameter and constriction. Our new screening tools will provide a precise measure of SFPN in a less invasive, more rapid approach to diagnosing small-fiber polyneuropathy in Veterans with Gulf War Illness.

15. SUBJECT TERMS: NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Gulf War Veterans report chronic multi-symptoms similar to that found in the treatable nerve disease small-fiber polyneuropathy (SFPN). Dr. Oaklander's preliminary evidence showed 47% of Gulf War Veterans had results consistent with SFPN. Evaluation for SFPN is expensive and lengthy. The aim of this research is to 1) develop screening tools for simple diagnosis by using patient-report symptom questionnaire and standardized medical exams, 2) develop biotechnology tools for simple diagnosis (sweat testing and pupilometry), 3) identify gene polymorphisms to detect risk for SFPN.

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

Polyneuropathy, Gulf War Illness, autonomic, skin biopsy, pupilometry, gene polymorphisms

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?

Goal 1: Human Research Approval		
a) Develop protocol, obtain IRB and Human Research Protection Office approvals	Oct 2015 - Dec 2015	**100% complete**
Goal 2: Recruitment		
a) Search existing veterans database, outreach to new veteran patients, advertisement at VA facilities, contact with VSOs, search of 30,000 veteran epidemiological study database, leverage other ongoing studies.	Dec 2015 - Mar 2018	**100% complete**
Goal 3: **Specific Aim 1**: To develop and evaluate screening tools for diagnosis and monitoring of longstanding eoSFPN, specifically a patient-reported symptom questionnaire and a standardized examination for medical personnel.		
a) Administer/validate questionnaires/forms	Sep 2016 - Mar 2018	**100% complete**
b) Data analysis and develop publications	Mar 2018 - Sep 2018	**80% complete**

Goal 4: **Specific Aim 2**: To develop and evaluate simple biotechnology devices for diagnosing and monitoring longstanding eoSFPN based on detection of abnormal sweating and pupil size and reactivity.		
a) Obtain supplies for skin biopsy	**Oct 2015 - Dec 2015**	**100% complete**
b) Study veterans with AFT and skin biopsy	**May 2016 - Mar 2018**	**100% complete**
c) Perform Sudoscan and pupillometry	**May 2016 - Mar 2018**	**100% complete**
d) Data analysis to determine sensitivity/specificity of Sudoscan and pupillometry	**Sep 2016 - May 2018**	**90% complete**
e) Publication of Sudoscan and pupillometry findings	**Mar 2018 - Sep 2018**	**0% complete**
Goal 5: **Specific Aim 3:** To develop and evaluate tools for identifying gene polymorphisms that convey risk for eoSFPN.		
a) Genetic sequence development	**Oct 2015 - Oct 2016**	**100% complete**
b) Blood draw for genetic materials	**Dec 2015 - Sep 2017**	**100% complete**
c) Genetic testing	**Sep 2016 - Sep 2017**	**50% complete**
d) Genetic data analysis	**Sep 2017 - May 2018**	**50% complete**
e) Publish genetic sequencing panel data	**Mar 2018 - Sep 2018**	**0% complete**

What was accomplished under these goals?

Major Activities:

1) Recruited and screened 91 veterans, of which 74 were eligible.
2) Collected AFT (n=40), skin biopsies (n=33), sudoscan (n=47) and pupilometry (n=41) data on veterans.
3) Collected blood from 46 veterans for genetic analysis.

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."

Staff were afforded the opportunity to go to the laboratory at Massachusetts General Hospital to learn how to perform Q-Sweat testing and SFPN physical examination properly from Drs Oaklander and Klein. During this collaborative visit our staff were able to demonstrate the testing for pupillometry capture as well. This has enhanced staff capabilities at both sites and will aid in the collection of accurate results.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Our initial findings were presented at the 29th International Symposium on Cerebral Blood Flow, Metabolism and Function:

1. **JM Serrador**, K Brewer, O Osinubi, M Klein, A Oaklander. Veterans with gulf war illness and small fiber neuropathy demonstrate worse cerebral blood flow regulation. The 29th International Symposium on Cerebral Blood Flow, Metabolism and Function. 2019. Yokohama, Japan.

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."

Nothing to report.

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."

The majority of the findings are reported in the final report submitted by Drs. Oaklander and Klein as part of the primary award.

Below is the initial findings from a subset of 28 veterans who were classified with regards to small fiber peripheral neuropathy (SFPN) and also had cerebral blood flow measures which were only obtained in Dr. Serrador's lab. To determine SFPN, a skin biopsy was performed. Densities <5th centile of predicted laboratory norms were categorized as SFPN+. Four participants were borderline and excluded from this analysis. GWI was determined using the Kansas questionnaire. Participant characteristics are in the table on the next page.

	GWI, SFPN-	GWI, SFPN+	P value
Sample size	*n* = 17	*n* = 7	
Nerve Fiber Density, %	64.5±20.4%	0.3±0.8%	<.001
Mean age, yrs	57 ± 7	53 ± 7	.260
Height, cm	173 ± 9	183 ± 7	.011
Weight, kg	89.7 ± 18.8	109.5 ± 18.0	.016
BMI	29.7 ± 6.6	32.6 ± 4.5	.209

Table showing GWI veteran characteristics in the Serrador lab that were classified as having small fiber peripheral neuropathy (SFPN+). Those without reduced nerve fiber density were classified as SFPN-.

Cerebral blood flow velocity of the middle cerebral artery (MCA) was measured using transcranial Doppler. In addition we obtained continuous blood pressure, heart rate, and end-tidal CO_2. To examine cerebral blood flow regulation, veterans performed three sit to stand maneuvers (3-min sitting, 1-min standing). To assess cerebrovascular response to changes in end-tidal CO_2, veterans breathed room air, followed by 8% CO_2, 21% O_2, balance nitrogen and then hyperventilated to reduce end tidal CO_2 ~10 mmHg.

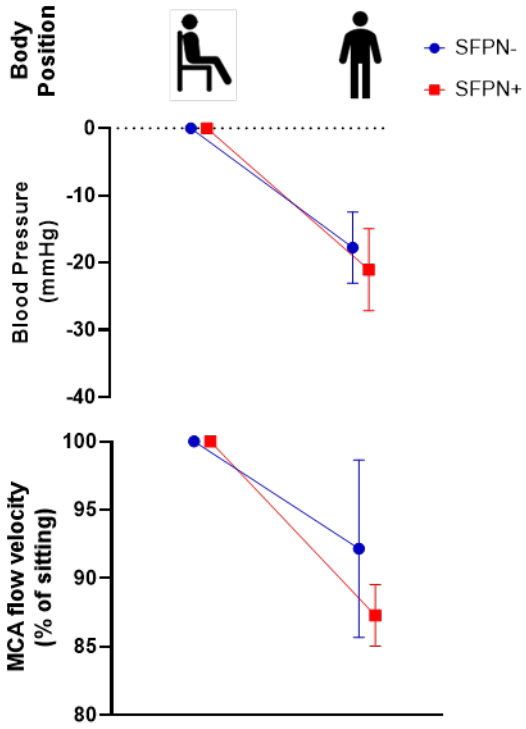

Figure 1 – Response to standing in veterans.

We found that upon standing (Fig. 1), veterans with SFPN+ and SFPN- demonstrated similar decreases in blood pressure (-21±6 vs -18±6 mmHg), however, MCA flow velocity was significantly lower in the SFPN+ group (87.3±2.2% of sitting baseline) compared to the SFPN- group (93.4±6.6%, P=0.05). We have previously found that veterans with GWI have impaired cerebral blood flow regulation and greater drops in cerebral flow velocity when standing. These data demonstrate that veterans with SFPN have even further decrements in their cerebral blood flow response, possibly contributing to the cognitive issues that veterans with GWI often report.

In addition, Figure 2 demonstrates that veterans with GWI that were SFPN+ demonstrated reduced autoregulation

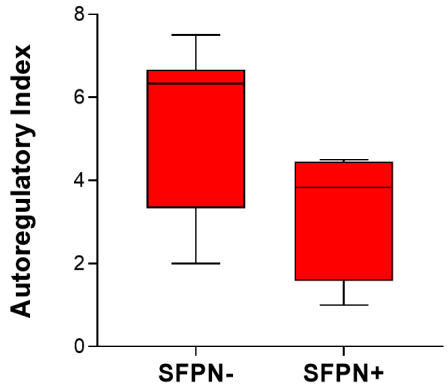

Figure 2 – Autoregulatory Index

index (3.3±1.6) compared to those that were SFPN- (5.9±1.7). This further supports that SFPN+ may affect cerebrovascular response. **This is a completely novel finding that has never been reported.** In fact, there is no literature in regards to either civilians or veterans showing the connection between SFPN and cerebral blood flow regulation.

Further evidence for a relationship between cerebral blood flow regulation and small fiber neuropathy is provided when we examine the relationship between nerve fiber density and autoregulation index, a measure of cerebral autoregulation. Figure 3 demonstrates the linear regression between autoregulatory index and nerve fiber density showed a significant positive correlation (R=0.498, P= 0.029). This indicates that veterans with GWI that have reduced nerve fiber density, even if they are not classified as having SFPN, still tend to have reduced autoregulatory index.

One surprising finding was that despite impaired cerebral autoregulation, which is the ability to maintain cerebral blood flow in the face of blood pressure changes, the inherent ability of cerebral vessels to dilate did not seem to be impaired. To test this vasodilatory ability, we examined change in cerebral blood flow when having veterans inspire increased levels of CO_2. Since CO_2 is a potent vasodilator we can examine how cerebral flow velocity changes with changes in end tidal CO_2. Figure 4 demonstrates that cerebrovascular reactivity to CO_2 was not significantly different between groups (SFPN- : 1.5±0.4 %/mmHg vs SFPN+ : 1.9±0.5 %/mmHg, P=0.10). In fact, it almost seems like veterans with SFPN+ had higher values, although not significant. So these data suggest there is no difference in the ability of cerebral vessels to dilate between veterans with and without SFPN.

We had originally hypothesized that veterans with GWI and SFPN+ would have dilated pupils under normal light conditions. We did pupil diameter measurements under three light conditions, bright lights to cause pupil constriction, normal room light levels and complete darkness to cause pupil dilation. Based on this, we found a tendency for increased

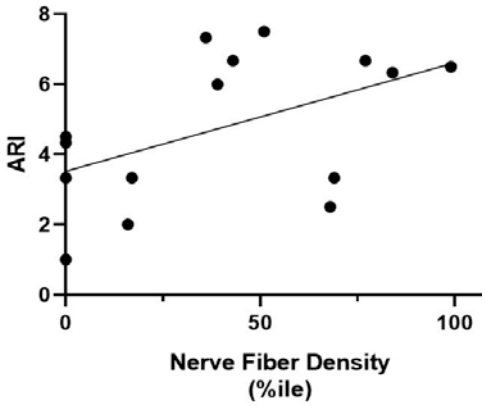

Figure 3 – Relationship between cerebral autoregulation and peripheral nerve density determined by skin biopsy.

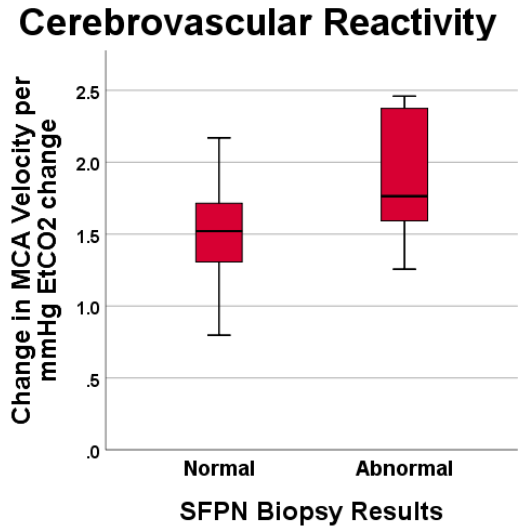

Figure 4 – Cerebral flow velocity changes in response to changing end tidal CO2 levels.

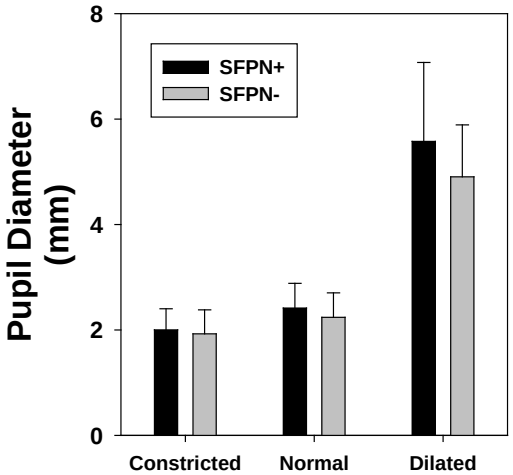

Figure 5 – Pupil diameters of veterans during bright lights (Constricted), normal room light (Normal) and in complete darkness (Dilated).

pupil diameter in the veterans with SFPN+ (Figure 5). However, this difference was not significant in the group of veterans we included in this analysis (SFPN-: N=8, SFPN+: N=13). We plan to redo the analysis once we have the complete dataset analyzed and determine if this trend continues to the point that it becomes significant.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

The finding that cerebral blood flow regulation may be impaired in the individuals with small fiber peripheral neuropathy could lead to new research in this area in this disease in civilians. This could provide new information on the pathophysiology of small fiber peripheral neuropathy and may indicate there is also a central component.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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."

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.

Nothing to Report

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 objectives at less cost than anticipated.

There were no changes in expenditures.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals.

No animal use research will be performed to complete the Statement of Work.

Significant changes in use of biohazards and/or select agents

No biohazards and/or select agents will be used to complete the Statement of Work.

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.*

Dr. Oaklander presented on the study and our collaborative efforts at the Gulf War Research Advisory Committee in April 2017.

Our initial findings were presented at the 29th International Symposium on Cerebral Blood Flow, Metabolism and Function:

JM Serrador, K Brewer, O Osinubi, M Klein, A Oaklander. Veterans with gulf war illness and small fiber neuropathy demonstrate worse cerebral blood flow regulation. The 29th International Symposium on Cerebral Blood Flow, Metabolism and Function. 2019. Yokohama, Japan.

- **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.

None.

- **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.

  None.

- **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;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *other.*

  None.

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: Jorge Serrador, PhD
Project Role: PI
Nearest person month worked: 2
Contribution to Project: no change

Name: Kelly Brewer, MS
Project Role: Study Coordinator
Nearest person month worked: 2
Contribution to Project: no change

Name: Leslie De La Cruz-Alvarez, BS
Project Role: Research Assistant
Nearest person month worked: 1
Contribution to Project: Leslie is performing data analysis.

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."

Nothing to Report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Organization Name: Massachusetts General Hospital
Location of Organization: Boston, MA
Partner's contribution to the project: Dr. Oaklander and Dr. Klein have visited VA NJ to provide expertise in scientific protocol development and train our staff in autonomic function testing.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

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.