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TITLE: Single Nucleus Expression Profiling of Human Sciatic Nerve After Traumatic

PRINCIPAL INVESTIGATOR: Thomas Van de Ven

CONTRACTING ORGANIZATION: Duke University, Durham, NC

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Thomas Van de Ven

E-Mail: thomas.vandeven@duke.edu

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Duke University
2200 W Main St, STE 710
Durham, NC 27708-4677

Uniformed Services University of the Health Science

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

A majority of service members who undergo traumatic amputation develop chronic phantom or chronic residual limb pain with 10-15% of these patients developing severe, disabling, long-term pain. 30-40% of traumatic amputees, however, have no clinically significant chronic pain. We believe this dichotomy of outcome is the key to understanding the development of chronic neuropathic pain after nerve injury. Preclinical studies using rodent models have provided some insights into the pathological sequelae of nerve injury, but this knowledge has not resulted in successful translation to the clinic. Recent evidence suggests that interspecies differences are a major barrier to successful translation, since rodent sensory neurons diverge considerably from their human counterparts. Accordingly, in order to better understand the pathological processes that lead to neuropathic pain after nerve injury, it is necessary to comprehensively study injured human nerves. Our colleagues at Walter Reed National Military Medical Center spent three years obtaining sciatic nerve samples from service members undergoing primary amputation revision surgery after suffering traumatic amputation on the battlefield. These unique samples allow, for the first time, study of nerve regeneration and neuroinflammation in humans during the days following traumatic amputation. Utilizing bulk tissue and single nuclei RNA-sequencing and unbiased global proteomics of the distal portion of sciatic nerve collected 1-14 days after initial traumatic amputation, we aim to establish the distinctive transcriptional, protein and glial/immune cell profile of injured sciatic nerve during injury and regeneration.

15. SUBJECT TERMS

Transcriptomics, single nuclei transcriptomics, proeteomics, neuroinflammation, neuropathic pain, nerve regeneration, phantom limb pain, residual limb pain.

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INTRODUCTION:

A majority of service members who undergo traumatic amputation develop chronic phantom or chronic residual limb pain with 10-15% of these patients developing severe, disabling, long-term pain. 30-40% of traumatic amputees, however, have no clinically significant chronic pain. We believe this dichotomy of outcome is the key to understanding the development of chronic neuropathic pain after nerve injury. Preclinical studies using rodent models have provided some insights into the pathological sequelae of nerve injury, but this knowledge has not resulted in successful translation to the clinic. Recent evidence suggests that interspecies differences are a major barrier to successful translation, since rodent sensory neurons diverge considerably from their human counterparts. Accordingly, in order to better understand the pathological processes that lead to neuropathic pain after nerve injury, it is necessary to comprehensively study injured human nerves. Our colleagues at Walter Reed National Military Medical Center spent three years obtaining sciatic nerve samples from service members undergoing primary amputation revision surgery after suffering traumatic amputation on the battlefield. These unique samples allow, for the first time, study of nerve regeneration and neuroinflammation in humans during the days following traumatic amputation. Utilizing bulk tissue and single nuclei RNA-sequencing and unbiased global proteomics of the distal portion of sciatic nerve collected 1-14 days after initial traumatic amputation, we aim to establish the distinctive transcriptional, protein and glial/immune cell profile of injured sciatic nerve during injury and regeneration.

KEYWORDS:

Transcriptomics, single nuclei transcriptomics, proeteomics, neuroinflammation, neuropathic pain, nerve regeneration, phantom limb pain, residual limb pain.

1. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

- **Aim 1 - Perform bulk tissue and single nuclei RNA-sequencing and unbiased global proteomics of the proximal and distal portion of sciatic nerve to establish the distinctive transcriptional, protein and glial/immune cell profile of injured sciatic nerve during injury and regeneration.**

- Major Task 1: Amend existing USUHS IRB and obtain approval for transcriptomic work on sciatic nerve samples. **100% complete (Performed at both Duke and USUHS/DVCIPM)**

    - This amendment was written by Dr. Van de Ven and submitted by Dr. Buckenmaier. After consultation with the IRB committee permission was given to sequence the RNA of these samples. This was a sensitive issue because the IRB at USUHS and Walter Reed are extremely protective of the genetic information of military subjects as is our group. Therefore we had to carefully describe and educate ourselves and the IRB about the genetic risks. Since transcriptomics use sequencing information only to measure transcript levels in cells, not to identify polymorphisms in the genetic code it is extremely unlikely that there will be genetic information obtained that would affect future care of the military servicemember or veteran. Also, there is no identifying information given to our group at Duke providing another layer of safety.

- Major Task 2: Obtain USAMRMC Office of Research Protections HRPO approval for use of sciatic nerve samples. **50% complete (Performed at Duke under Dr. Vandeven)**
    - All IRB documents were submitted for HRPO approval and we are working with the Office of Research Protections now to obtain this approval.
- Major Task 3: Renew IRB exemption for work on deidentified nerve samples at Duke. **100% complete (Performed at Duke under Dr. Vandeven)**
    - This exemption no longer has an expiration date
- Major Task 4: Process each nerve, collect nuclei and bulk RNA. **10% complete (Performed at Duke under Dr. Vandeven)**
    - After submission of this grant application but before the funding announcement we used the existing limited IRB approval from USUHS, Walter Reed and Duke to process one non-SEXI study nerve and one duplicate nerve from this study. We performed a number of different processing techniques and found that cryotome slicing the nerve samples produced the most consistent sized samples, allowed transcriptomics and proteomics from slices very spatially close to each other making comparisons of protein and RNA expression much more accurate, and allowed processing without complete thawing.
- Major Task 5: Create RNA libraries and complete sequencing. **5% complete (Performed at Duke under Dr. Vandeven)**
    - Though we could not perform this experiment without IRB and HRPO approval we did spend a lot of time setting ourselves up for success. We spoke with the UNC and Duke sequencing core facilities and the Duke bioinformatics core to discuss study design. We also have settled on a protein and nuclei extraction protocol that worked well on test samples.
- Major Task 6: Complete proteomics. **5% complete (Performed at Duke under Dr. Vandeven)**
    - We have had multiple discussions with the Duke Proteomics Core facility outlining a very interesting study plan that will not only look at protein signatures at one location in each nerve but will take multiple samples in succession to look at protein signature changes along the nerve as samples get farther away from the injured end. We can also do a temporal analysis of protein signature since samples were collected at a range of timepoints from the date of injury.
- Major Task 7: Develop an expression and cell signature of nerve regeneration over the two weeks following traumatic amputation. **0% complete**
- **Aim 2 - Complete a pain and functional outcome database describing each patient enrolled in the SEXI trial and use those outcomes to identify immune cell populations, gene and protein expression changes around the time of amputation that correlate with positive outcomes of good function and minimal residual limb or phantom pain.**
- Major Task 1: Amend existing USUHS IRB to collect physical function and more recent pain and analgesic medication data on the patients who previously donated sciatic nerve samples. **100% complete (performed by the USUHS/DVCIPM team under Dr. Buckenmaier)**

- Major Task 2: Update current clinical outcomes database of SEXI patients for most recent pain scores and medication use (60 total patients) **25% complete (performed by the USUHS/DVCIPM team under Dr. Buckenmaier)**
    - Between the time of grant submission and award we already had IRB approval to gather limited information on pain scores and the SEXI database was updated with that information.
- Major Task 3: Add functional outcomes data to database. **0% complete**
- Major Task 4: Correlate outcomes with expression signature **0% complete**
- **Aim 3 - Correlate perioperative ketamine use with immune cell population and gene and protein expression profile.**
- Major Task 1: Determine whether ketamine treatment produces improved functional outcomes. **0% complete**
- Major Task 2: Identify the protein and RNA expression signature in sciatic nerve unique to patients treated with perioperative ketamine. **0% complete**
- **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 yet
- **What do you plan to do during the next reporting period to accomplish the goals?**
    - During the next reporting period all samples will have been processed, sent to one of three core facilities for proteomics or transcriptomics. Also, complete clinical data will be collected at Walter Reed and USUHS. The reporting period after that will be used for data analysis.

2. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**
    - Nothing to Report yet
- **What was the impact on other disciplines?**
    - Nothing to Report yet
- **What was the impact on technology transfer?**
    - Nothing to report
- **What was the impact on society beyond science and technology?**
    - Nothing to report

3. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**
    - Though we have not encountered any roadblocks to completing the scientific aims of this study, we have explored different mechanisms to get the work done. The UNC sequencing core facility has extensive experience performing single nuclei sequencing and with the departure of Dr. Qadri from Duke we plan to use this expertise to complete this portion of the project. We will submit a budget amendment detailing these changes.
- **Actual or anticipated problems or delays and actions or plans to resolve them**
    - It took longer than anticipated to get IRB approval for sequencing these samples. Also, our lab was shut down and is still limited by COVID precautions. However, we have done a significant amount of preparatory work which will make completion of this project within the original time span very likely.
- **Changes that had a significant impact on expenditures**
    - The departure of Dr Qadri and the research pause due to COVID-19 made me explore other avenues to cost-effectively carry out the research described in the proposal. I met with the UNC sequencing core who can take the nuclei I extract and perform single nuclei sequencing for less cost than it would have been to do it using Dr. Qadri, a research technician and Duke core facilities. I will submit a budget amendment with specifics but my hope is the same quality work can be performed for the same and likely less money.
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
    - We are awaiting HRPO approval but did obtain Walter Reed and USUHS approval for the transcriptomics sections of this project
- **Significant changes in use or care of human subjects**
- **Significant changes in use or care of vertebrate animals.**
- **Significant changes in use of biohazards and/or select agents**

4. **PRODUCTS:**

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

Journal publications.

Nothing to Report yet

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

Nothing to report

Other publications, conference papers, and presentations.

Nothing to report

5. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**

Name:	*Thomas Van de Ven*
Project Role:	*Principal Investigator*
Researcher Identifier (eRA Comm	*THOMAS.VANDEVEN*
Nearest person month worked:	*6*
Contribution to Project:	*Coordinates all aspects of the project and assumes overall responsibility for its success.*
Funding Support:	*No other support*

Name:	*Yawar Qadri*
Project Role:	*Co-investigator*
Researcher Identifier (eRA Commons):	YQadri
Nearest person month worked:	*4.8*
Contribution to Project:	*Coordinates single nuclei sequencing aspect of the project. Dr. Qadri left Duke this summer and his effort will be replaced by the UNC single cell sequencing core facility.*
Funding Support:	*No other support*

Name:	*Chester Buckenmaier*

Project Role:	*Site Prinicipal Investigator*
Researcher Identifier (eRA Comm	cbuckenmaier
Nearest person month worked:	*0.24*
Contribution to Project:	*Coordinates IRB approval of study activities and collection of clinical data on enrolled subjects*
Funding Support:	*American Massage Therapy Association, Uniformed Services University (4), DoD (2)*

Name:	*Mary McDuffie*
Project Role:	*Research Coordinator*
Researcher Identifier (eRA Commons):	MARYMCDUFFIE
Nearest person month worked	*1.44*
Contribution to Project:	*Responsible for providing research management, administrative support, and project coordination to include protocol management, regulatory affairs, effective use of project resources, purchasing, meetings, travel.*
Funding Support:	*DoD*

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

- **Organization Name:** Defense and Veterans Center for Integrative Pain Management (DVCIPM) and Uniform Services University of the Health Sciences (USUHS)
- **Location of Organization:** Bethesda, Maryland
- **Partner's contribution to the projec**t

Collaboration Dr Buckenmaier and the research staff at DVCIPM are responsible for IRB approval of this study and for collection of the clinical data needed to tie molecular changes in the sciatic nerve samples to important clinical functional and pain outcomes

6. **SPECIAL REPORTING REQUIREMENTS**

- **COLLABORATIVE AWARDS:**
- **QUAD CHARTS:**

7. **APPENDICES:**