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TITLE: Does TBI Affect mtDNA Heteroplasmy?

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CONTRACTING ORGANIZATION: University of Kansas Medical Center

Shawnee Mission, KS
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Russell H. Swerdlow, M.D.

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14. ABSTRACT

This collaborative project seeks to better understand the long-term consequences of traumatic brain injury (TBI) as they pertain to Alzheimer's disease (AD). We propose that a traumatic brain injury (TBI) accelerates the age-related accumulation of mitochondrial DNA (mtDNA) microheteroplasmic mutations, and that this will explain the recognized association between TBI and Alzheimer's disease (AD). The project's specific aims are (1) to test whether a young adulthood controlled cortical injury (CCI) accelerates the age-dependent accumulation of mtDNA mutations, (2) to test whether brain mtDNA heteroplasmy in aged mice subjected to a young adulthood CCI injury relates to behavioral function, brain metabolism, and brain structure, and (3) to test the relationship between age-related accumulation of mtDNA mutations and neurofibrillary tangles in a genetically engineered strain of mice (rTg4510). We anticipate that findings from this study will help to explain why sustaining a TBI during young adulthood increases one's late-life risk of developing AD.

15. SUBJECT TERMS
Traumatic Brain Injury, Alzheimer's Disease, Aging, Mitochondrial DNA, Mutations, Functional impairment, Memory, Magnetic resonance imaging, Magnetic resonance spectroscopy, Tau pathology

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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TABLE OF CONTENTS

INTRODUCTION:

This collaborative project seeks to better understand the long-term consequences of traumatic brain injury (TBI) as they pertain to Alzheimer's disease (AD). To accomplish this, we will explore connections between TBI, brain aging, and mitochondrial DNA (mtDNA) mutations. Our central hypothesis is that TBI will accelerate the age-related accumulation of mtDNA microheteroplasmic mutations, and that this will explain the recognized association between TBI and AD. To test this, we will expose young adult mice to a TBI or sham intervention and let the mice age. In Aim 1, we will determine whether the TBI sustained during young adulthood accelerates the age-related accumulation of mtDNA heteroplasmy. In Aim 2, we will characterize these aging, brain-injured mice through behavioral testing, magnetic resonance imaging and spectroscopy, and measurements of mitochondrial enzyme activity, and we will correlate mtDNA heteroplasmy with these endpoints. In Aim 3, we will administer CCI to young adult rTg4510 mice (which accumulate neurofibrillary tangles), age the mice, quantify mtDNA heteroplasmy and neurofibrillary tangle burden, and test relationships between these two parameters. We anticipate that successful completion of these studies will help to explain why sustaining a TBI during young adulthood increases one's late-life risk of developing AD.

1. **KEYWORDS:**

Traumatic Brain Injury
Alzheimer's Disease
Aging
Mitochondrial DNA
Mutations
Functional impairment
Memory
Magnetic resonance imaging
Magnetic resonance spectroscopy
Tau pathology

2. **ACCOMPLISHMENTS:**

The major goals of the project are:

Aim 1: Test whether a young adulthood CCI injury accelerates the age-dependent accumulation of mtDNA.

Aim 2: Test whether brain mtDNA heteroplasmy in aged mice subjected to young adulthood CCI injury relates to behavioral function, brain metabolism, and brain structure.

Aim 3: Test whether neurofibrillary tangle burden in aged rTg4510 mice subjected to young adulthood CCI injury relates to mtDNA mutations.

The activities for this reporting period (months 13-24) are:

Aim 1:

Subtask 1 (months 1-3): Local IACUC Review/approval – COMPLETE.
Subtask 2 (months 4-6): ACURO Review/approval – COMPLETE.
Subtask 3 (months 6-10): Acquire mice, acquisition will be staggered – COMPLETE in month 13.
Subtask 4 (months 7-11): Administer CCI – COMPLETE in month 15.
Subtask 5 (Months 8-12): Sacrifice mice & collect DNA 1 month after CCI – COMPLETE in month 16.
Subtask 6 (Months 8-26): Age the mice – began in month 11, ongoing.

**We note that the timeline for Subtasks 3-6 was delayed due to a mouse parvovirus outbreak in year 1, as detailed in previous technical reports.*

Aim 2:

Subtask 1 (months 1-3): Local IACUC Review/approval – COMPLETE.
Subtask 2 (months 4-6): ACURO Review/approval – COMPLETE.
Subtask 3 (months 6-10): Acquire mice, acquisition will be staggered – COMPLETE in month 13.
Subtask 4 (Months 7-11): Administer CCI – COMPLETE in month 15.
Subtask 5 (Months 7-26): Assess time course of cognitive and motor function before and after CCI – began in month 10, ongoing.
Subtask 6 (Months 8-26): MRI/MRS 1 and 15 months after CCI – began in month 11, ongoing.
Subtask 7 (Months 8-26): Age the mice – began in month 11, ongoing.
Subtask 8 (Months 8-26): Sacrifice mice & collect brain hemispheres 1 and 15 months after CCI – began in month 11, ongoing.
Subtask 9 (Months 8-26): Perform mitochondrial enzyme assays – began in month 19; ongoing.

**We note that the timeline for Subtasks 3-9 was delayed due to a mouse parvovirus outbreak in year 1, as detailed in previous technical reports.*

Aim 3:

Subtask 1 (months 1-3): Local IACUC Review/approval – COMPLETE.
Subtask 2 (months 4-6): ACURO Review/approval – COMPLETE.
Subtask 3 (by month 8): Acquire mice, acquisition will be staggered – COMPLETE in month 16.
Subtask 4 (by month 9): Administer CCI – COMPLETE in month 17.
Subtask 5 (months 10-24): Age the mice – began in month 17, ongoing.
Subtask 6 (by month 24): Collect hippocampus DNA 15 months following CCI – now scheduled for month 32.
Subtask 7 (by month 24): Prepare frontal cortices for tau tangle analysis – now scheduled for month 32.
Subtask 8 (by month 29): Sequence and analyze mtDNA – now scheduled for months 32-36.
Subtask 9 (by month 29): Tangle analysis – now scheduled for months 32-36.

**We note that the timeline for Subtasks 3-9 was delayed due to limited availability of the transgenic mice from the vendor, as detailed in previous technical reports.*

Accomplishments under these goals:

1) Major activities:
- Completed surgical, behavioral, and neuroimaging studies through the 1 month time point in all c57Bl/6 mice.
- Completed surgical and neuroimaging studies through the 1 month time point in all rTg4510 mice.
- Euthanized the 1-month survival mice and collected brain samples for mtDNA and mitochondrial enzyme analyses.
- Refined methods for mtDNA sequencing
- Developed post-acquisition data processing and correction methods for multi-voxel MRS imaging in mouse brain.
- Monitored health of the long-term survival mice; maintained detailed health records.
- Completed preliminary analyses of the MRI and behavioral data collected to date.

2) Specific objectives:
- Complete all CCI/Sham surgeries
- Complete post-surgery behavioral and imaging assessments through the 1 month timepoint
- Euthanize the 1 month survival mice and collect brain tissue samples
- Begin aging the long-term survival mice
- Process and analyze data collected through the 1 month timepoint

Discussion of delay in our experimental timeline:
As noted above, for each aim of our project certain subtasks were affected by unexpected delays in year 1. These have been previously described in quarterly technical reports. In brief, aspects of Aims 1 and 2 were delayed by a parvovirus outbreak in our University's animal facility. Aim 3 was delayed by limited availability of the rTg4510 mouse strain from the vendor.

3) Significant results or key outcomes: The figures below present preliminary data analyses we have generated during year 2 of the study.

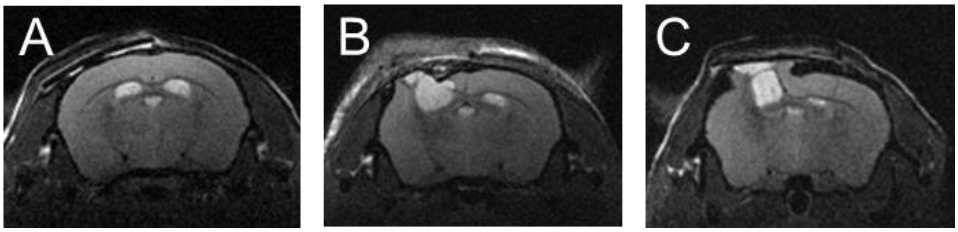

Figure 1. Example T2-weighted magnetic resonance images acquired 1 month post-surgery in a c57 sham mouse (A), c57 TBI mouse (B), and rTg4510 TBI mouse (C).

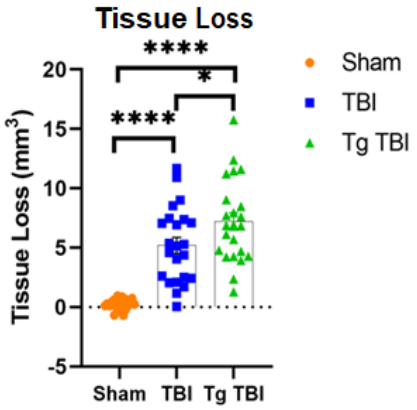

Figure 2. Quantitative lesion analysis in magnetic resonance images acquired 1 month post-surgery revealed an average brain tissue loss of 0.24 ± 0.09 mm^3 in Sham mice, 5.20 ± 0.66 mm^3 in wild-type TBI mice, and 7.19 ± 0.73 mm^3 in transgenic TBI mice.

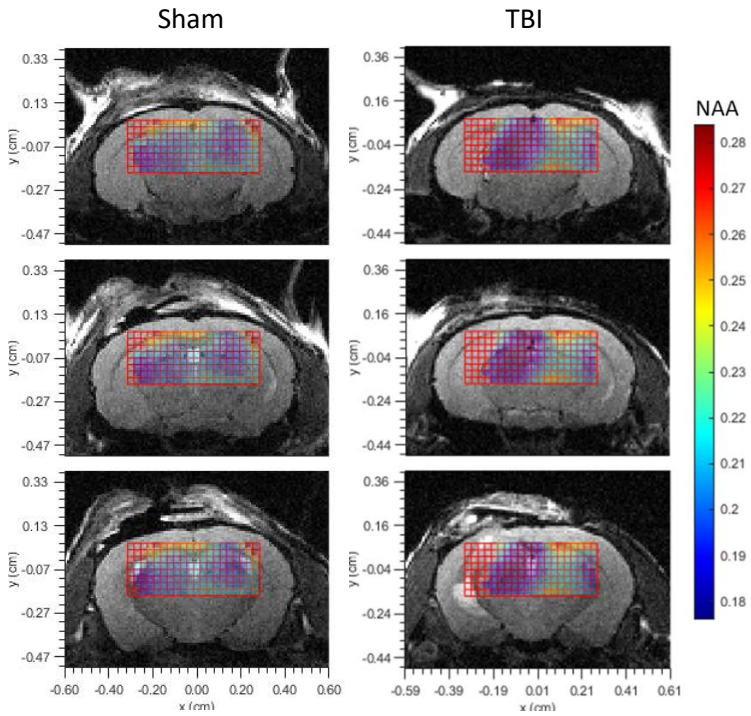

Figure 3. Multi-voxel Chemical Shift Imaging (CSI) spectroscopic acquisition. During the reporting period we optimized this imaging technique for use in mice after TBI, and developed post-acquisition processing and corrections methods. Example CSI maps are shown overlaid on T2-weighted images for a Sham mouse (left) and TBI mouse (right) at 1 month post-procedure. Quantitative analyses are ongoing to evaluate metabolites by regions of interest from the ipsi-lesional and contra-lesional brain hemispheres.

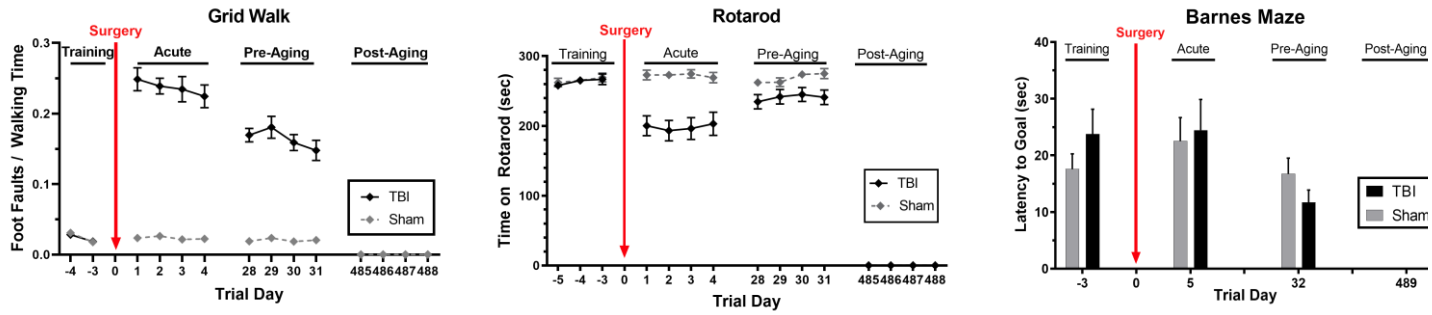

Figure 4. Preliminary analyses of performance on on our battery of behavioral tests through 1 month post-TBI/Sham. TBI mice showed significant deficits on the Grid Walk and Rotorod tasks compared to Sham controls. The groups did not differ in performance on the Barnes Maze task. Mice are aging and will be tested again on the behavioral tasks at 15 months post-TBI/Sham (day 485-488).

4) Other achievements:

- Bimonthly meetings between the Co-PIs for discussion, updates, and strategic planning.
- Frequent (at least monthly) meetings with key hands-on personnel involved in animal studies.

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

Nothing to Report.

How were the results disseminated to communities of interest?

Preliminary results were presented by Dr. Harris in invited talks at:

1) Virginia Commonwealth University Department of Anatomy and Neurobiology (Feb 2020)
2) University of Kentucky Current Topics in TBI webinar series (July 2020)

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

During the next reporting period, we will age the mice until they reach 15 months post-TBI/sham, then perform imaging and behavioral studies and euthanize to collect brain tissues. On the collected hippocampal samples we will perform mtDNA sequencing and analyze results to quantify mtDNA heteroplasmy. We will process the frontal cortical samples for stereological analysis of tau tangles. We will assess the parietal cortical samples using mitochondrial enzyme activity assays.

Once data collection is complete for all mice in the study, we will proceed with analyzing relationships between the study measures to test our overarching hypothesis that TBI accelerates the age-related accumulation of mtDNA mutations. We will collaboratively interpret results and prepare our findings for dissemination.

3. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?
Nothing to Report.

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.

4. **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
In March-May 2020 our institution imposed a mandatory shutdown of all research activities due to COVID-19. However, animals from our study were allowed to be cared for and maintained in university husbandry facilities. The actual impact of the shutdown on our study was minimized by fortunate timing, since we had few in-lab experiments planned for this period and were primarily letting the mice age. Working from home during the shutdown, we were able to continue with processing and analysis of the data we had previously collected.

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
Nothing to Report.

Significant changes in use or care of human subjects
N/A

Significant changes in use or care of vertebrate animals.
Nothing to Report.

Significant changes in use of biohazards and/or select agents
N/A

5. **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.**
  Preliminary results were presented by Dr. Harris in invited talks at:
  1) Virginia Commonwealth University Department of Anatomy and Neurobiology (Feb 2020)
  2) University of Kentucky Current Topics in TBI webinar series (July 2020)
- **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**
  Nothing to Report.

6. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

What individuals have worked on the project?

Name:	Janna Harris
Project Role:	Multiple PI (Contact)
Research Identifier:	0000-0002-4829-6360
Nearest person month worked:	3.7 Calendar Months
Contribution to Project:	Dr. Harris has coordinated work by the study team to carry out all live animal experiments. She has supervised scheduling to keep the timeline on track, overseen data analysis, and coordinated meetings with co-PI Dr. Swerdlow and with other individuals on the project.
Funding Support:	No change

Name:	Russell Swerdlow
Project Role:	Multiple PI
Research Identifier:	0000-0003-2948-7230
Nearest person month worked:	1.2 Calendar Months
Contribution to Project:	Dr. Swerdlow has worked collaboratively with co-PI Dr. Harris and the study team to supervise execution of the study protocols, to review the progress of animal experiments, and to troubleshoot when issues arise.
Funding Support:	No change

Name:	Xiaowan Wang
Project Role:	Postdoctoral Fellow
Research Identifier:	Not applicable

Nearest person month worked:	4.4 Calendar Months
Contribution to Project:	Dr. Wang has carried out the mouse behavioral testing, euthanasia and brain tissue dissections, and has worked to refine protocols for the mitochondrial enzyme assays.
Funding Support:	No change

Name:	Judit Perez-Ortiz
Project Role:	Postdoctoral Fellow
Research Identifier:	0000-0002-8953-4281
Nearest person month worked:	4.7 Calendar Months
Contribution to Project:	Dr. Perez-Ortiz has carried out the mouse behavioral testing and data analysis, euthanasia and brain tissue dissections, and assisted with stereology protocols.
Funding Support:	No change

Name:	(Harry) Scott Barbay
Project Role:	Senior Scientist
Research Identifier:	0000-0003-2470-7775
Nearest person month worked:	3.0 Calendar Months
Contribution to Project:	Dr. Barbay assisted with preparation of the animal protocols and sourcing of research supplies. During the reporting period he completed 73 TBI/sham surgeries and assisted with ongoing animal health monitoring and medical records.
Funding Support:	No change

Name:	Sarah Christian
Project Role:	Research Assistant
Research Identifier:	Not applicable
Nearest person month worked:	9.0 Calendar Months
Contribution to Project:	Ms. Christian assisted with all TBI/sham surgical procedures, MRI scans, and animal behavior assessments. She has also been responsible for sourcing and ordering research supplies, monitoring the health of the mice, and maintaining study records and laboratory compliance.
Funding Support:	No change

Name:	DongWei Hui
Project Role:	Senior Research Associate
Research Identifier:	0000-0003-1020-8940
Nearest person month worked:	2.3 CYM
Contribution to Project:	Dr. Hui refined methodologies for mitochondrial DNA isolation from mouse brain and for deep sequencing without contamination from nuclear DNA.
Funding Support:	No change

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

Swerdlow, Russell:

AWARDED
R01 AG062548-01A1 (Morris, Jill Kathleen) 02/15/2020 - 01/31/2025 0.48 CM
NIH $317,407
Acute exercise and the cerebral metabolic response in aging and Alzheimer's Disease
Major Goals: To characterize cerebral glucose metabolism in response to acute exercise. To compare the effect of different exercise intensities. To determine differences in the biomarker response between diagnosis groups
Role: Co-Investigator

R01 AG064227-01A1 (Slawson, Chad E) 04/01/2020 - 03/31/2025 1.17 CM
NIH $439,720
O-GlcNAc Homeostasis Regulates Mitochondrial Function In Alzheimer's Disease
Major Goals: Understand how alterations in O-GlcNAc contribute to the onset and progression of AD.
Role: Co-Investigator

R21 AG058052-01A1 (Harris, Janna Leigh) 09/01/2019 - 05/31/2021 0.36 CM
NIH $125,000
Bioenergetic Dysfunction in the Aging, Injured Brain: Characterization and Intervention with Creatine
Major Goals: 1) Characterize the effects of aging on the bioenergetic sequelae of traumatic brain injury 2) Compare effects of bioenergetic manipulation with creatine supplementation in the young and aged, injured brain.
Role: Co-Investigator

Harris, Janna:

AWARDED
R21 AG058052-01A1 (Harris, Janna Leigh) 09/01/2019 - 05/31/2021 2.4 CM
NIH $125,000
Bioenergetic Dysfunction in the Aging, Injured Brain: Characterization and Intervention with Creatine
Major Goals: 1) Characterize the effects of aging on the bioenergetic sequelae of traumatic brain injury 2) Compare effects of bioenergetic manipulation with creatine supplementation in the young and aged, injured brain.
Role: Principal Investigator

Brooks, William:

AWARDED
R21 AG058052-01A1 (Harris, Janna Leigh) 09/01/2019 - 05/31/2021 0.36 CM
NIH $125,000
Bioenergetic Dysfunction in the Aging, Injured Brain: Characterization and Intervention with Creatine
Major Goals: 1) Characterize the effects of aging on the bioenergetic sequelae of traumatic brain injury 2) Compare effects of bioenergetic manipulation with creatine supplementation in the young and aged, injured brain.
Role: Co-Investigator

Nudo, Randolph:

AWARDED
<NONE> (Tuchek, Chad Alexander) 7/01/2020 - 06/30/2021 0.3 CYM
Neurosurgery Research and Education Foundation; $20,000
Evaluation of Novel Polymeric Substrates for Direct Extradural Application of Therapies Following Traumatic Brain Injury: An Innovative Approach to a Difficult Problem
Major Goals: Aim 1: To assess the neurobehavioral effects and recovery when a novel dexamethasone "bone paste" is applied to the cranial defect in a rat TBI model. Aim 2a will utilize high resolution MRI to assess cytoarchitectural and white matter tract changes following application of a novel cranioplasty material and dexamethasone in TBI rats Aim 2b: To assess osteogenic properties of the hydrogel bone paste following hemicraniectomy
Role: Co-Investigator

What other organizations were involved as partners?
Nothing to Report.

7. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:**
     Not applicable.
   - **QUAD CHARTS:**
     An updated quad chart is provided as a separate attachment.

8. **APPENDICES:**
   None.