AWARD NUMBER: W81XWH-20-1-0281

TITLE: Impact of APOE Genotype on Astrogliopathies in Repetitive mTBI

PRINCIPAL INVESTIGATOR: Drs. Fiona Crawford

CONTRACTING ORGANIZATION: The Roskamp Institute, Inc.
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PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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1. REPORT DATE: OCTOBER 2021	**2. REPORT TYPE: Annual**	**3. DATES COVERED: 09/15/2020 – 09/14/2021**

4. TITLE AND SUBTITLE:

Impact of APOE Genotype on Astrogliopathies in Repetitive mTBI

5a. CONTRACT NUMBER
W81XWH-20-1-0281

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Fiona Crawford, PhD – Contact PI; Joseph Ojo, PhD – Co-PI; Lauren Horne – Grant Coordinator

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES):
The Roskamp Institute, Inc., 2040 Whitfield Avenue Sarasota, FL 34243-3922

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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13. SUPPLEMENTARY NOTES: N/A

14. ABSTRACT: Repetitive mild TBI (r-mTBI) is an environmental risk factor for Alzheimer's Disease (AD) and related dementia (ADRD). One of the hallmark features of r-mTBI is the localization of tau in astrocytes, termed tau astrogliopathies. This feature is also observed in advanced aging (ARTAG) and in FTD-Tau brains. The precise nature of how TBI leads to or precipitates this path towards astrogliopathy remains elusive. APOE genotype, particularly the APOE4 allele, is a risk factor for sporadic ADRD cases such as AD and FTD, and is linked with increased risk for ADRD after TBI. ApoE is produced by astrocytes in the brain, but its role in promoting astrogliopathies in the context of TBI remains unknown. We have generated molecular profiles of mTBI pathogenesis in r-mTBI models, collected at a range of timepoints post-injury. Our data clearly demonstrate the unique contributions from different cell types, particularly reactive astrocyte pathologies, and the critical role for microglia inflammation. Our preliminary data also suggest that APOE genotype differentially influences TBI-induced astrocyte and microglia pathobiology, and this correlates with detection of pathogenic neuronal tau species in APOE4-targeted replacement (TR) mice, and those crossed with hTau knock-in mice following r-mTBI. However, none of our models (or any in the literature) has clearly demonstrated TBI dependent tau astrogliopathy. In this proposal, we plan to utilize APOE-TR mice engineered to produce non-mutant hTau in astrocytes (E2/E3/E4*GFAP-Tau), to enable us to explore the influence of APOE genotype on TBI-dependent astrogliopathy. *We hypothesize that astrocytes are key to the pathobiological sequelae and lesions observed in TBI and ADRD, and this can be influenced by APOE genotype. Administering r-mTBI to mice expressing human APOE genotypes and non-mutant hTau in astrocytes will induce pathological lesions in reactive astrocyte populations, which will be more severe in APOE4 versus APOE3 or APOE2 mice, contributing to the pronounced spreading of proteinopathies and mediation of cell and non-cell autonomous neurodegeneration that is driven by reactive microglia. In Aim 1 we will, delineate the influence of APOE genotype on pathological and biochemical outcomes 3 and 6 Mos after r-mTBI/sham in a GFAP-hTau^{WT} mouse model with astrocyte specific (non-mutant) hTau expression. In Aim 2 we will, delineate the contribution of astrocytes to the spreading of Tau proteinopathies, and subsequent neurodegeneration post-TBI, and how this is influenced by APOE genotype. In Aim 3 we will, delineate the contribution of microglia in driving TBI-induced Tau astrogliopathies, and how this is influenced by APOE genotype.*

15. SUBJECT TERMS: TBI, ADRD, APOE, Tau Astrocytes, Neuropathology, Transcriptomics, Single cell profiling, Animal models

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	16	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION: ***Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.***

In this proposal, we plan to utilize APOE-TR mice engineered to produce non-mutant hTau in astrocytes, to enable us to explore the influence of APOE genotype on TBI-dependent astrogliopathy. We will use our novel mechanistic approaches involving adoptive cell transfer and *ex vivo* functional assays to clarify the means through which APOE4 genotype and astrocytes contribute to TBI-neurodegeneration and precipitation of tau astrogliopathy. We will conclude by applying our state of the art single cell gene profiling to interrogate astrocytes from our mouse models and autopsy cases from different APOE genotype backgrounds to identify astrocyte specific changes at the gene and protein level, and to identify novel single cell targets to interrupt the pathobiological consequences of TBI.

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

TBI, ADRD, APOE, Astrocytes, Tau, Neuropathology, Transcriptomics, Single cell profiling, Animal models.

3. ACCOMPLISHMENTS: *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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.

SOW Task	Timeline (completion)	Progress
MAJOR TASK 1: *We will delineate the influence of APOE genotype on pathological and biochemical outcomes 3 and 6 Mos after r-mTBI/sham in a GFAP-hTau^{WT} mouse model with astrocyte specific (non-mutant) hTau expression.*		
Subtask 1a: Obtaining ACURO approval (Roskamp Institute approval for TBI procedures is already in place).	Y1Q1	Completed
Subtask 1b: Obtaining Human Cadaver use approval.	Y1Q1	Completed
Substask 2: Breeding of cohorts for injuries (24 mice per genotype hAPOE2, hAPOE3, hAPOE4, hAPOE2*GFAP-Tau, hAPOE3* GFAP-Tau, hAPOE4*GFAP-Tau) at 3 & 6 months post-TBI	Y1Q2	In progress, 50%
Subtask 3: Administering injuries or sham injuries at 3 months of age. Animals will receive 2 closed head injuries each week for 3 months, using a CCI device. There is will 6 genotypes, 12 cr-mTBI and 12 cr-sham mice per group. A total of 144 mice will be used (72 cr-mTBI and 72 mice r-sham).	Y1Q2 to Y1Q4	Not started (behind schedule)
Subtask 4: Tissue harvest of the 3 months cohort from subtask 3.	Y2Q1	Not started
Subtask 5: Tissue harvest of the 6 months cohort from subtask 3.	Y2Q2	Not started
Subtask 6: Sectioning, histopathological staining/analyses, and biochemical analyses of mouse brain tissues analyzed. N=144 brains will be analyzed and staggered over time.	Y1Q4 to Y2Q3	Not started
Subtask 7: Interpretation of data/consultation with neuropathologists	Y2Q3	Not started
MAJOR TASK 2: *We will delineate the contribution of astrocytes to the spreading of Tau proteinopathies, and subsequent neurodegeneration post-TBI, and how this is influenced by APOE genotype.*		
Subtask 1: Laser capture microdissection (LCD) and single cell analyses of astroglia from autopsy. Cases will consist of 16 Groups of ARTAG, FTD-Tau, and r-mTBI brains and age-matched controls, with each disease/control group consisting of APOE3/E3 and APOE4/E4. N=8/group; 128 total samples. Samples are coded and de-identified.	Y1Q2 to Y2Q1	In progress 30%

Subtask 2: Breeding of cohorts for Young Donor mice for TBI studies in Subtask 4 and 6 (12 mice per genotype for hAPOE2*GFAP-Tau, hAPOE3*GFAP-Tau, hAPOE4*GFAP-Tau)	Y1Q1 to Y1Q2	In progress, 50%
Subtask 3: Administering injuries to Young 3Mos old 'Donor' cohort. Animals will receive injuries as in Major task 1 (subtask 3). 36 total mice - 6 cr-mTBI/6 cr-sham per genotype.	Y1Q4	Not started
Subtask 4: Isolation of astrocytes from 'Young Donor' mouse cohort and injection into 'Recipient' mouse brain. Recipient mice will consist of 42 total C57BL6 mice (14 mice per day).	Y2Q2	Not started
Subtask 5: Inoculation of recipient mice with astrocyte derived tau from human FTD-Tau, ARTAG, TBI, and aged matched control brains (Change to mouse tissue from GFAP-P301L mice or AAV). N= 72 recipient APOE-TR mice (24 per genotype).	Y2Q3	Not started
Subtask 6: Proteomic response in healthy neural/ microvascular cells exposed to secretomes of APOE-TR and tau bearing primary astrocytes cultures from models used in Major Task 1.	Y2Q3	Not started
Subtask 7: Tissue harvest, sectioning, histopathological staining and analyses of inoculated recipient mice from Major Task 2 (subtask 4 & 5). N=114 mice total.	Y2Q3 to Y2Q4	Not started
Subtask 8: LCD and single cell analyses of astroglia from mice in Major Task 1 (subtask 3). 24 mouse brains per genotype for analyses. (N=72 mouse brains total)	Y2Q2 to Y2Q4	Not started
MAJOR TASK 3: *We will delineate the contribution of microglia in driving TBI-induced Tau astrogliopathies, and how this is influenced by APOE genotype.*		
Subtask 1: LCD of microglia from 128 autopsy brains (in Major Task 2, subtask 1) for single cell gene expression profiling.	Y1Q4	Not started
Subtask 2: Breeding of cohorts for Donor mice in subtask 5.	Y1Q4	Not started
Subtask 3: Administering injuries to 'Donor' cohort - 3 months old injury. Animals will receive injuries as in Major task 1 (subtask 3).	Y2Q3	Not started
Subtask 4: Microglia ablation studies using PLX3397 administration. 72 hAPOE-TR*GFAP-Tau^{WT} mice.	Y2Q3 to Y2Q4	Not started
Subtask 5: Isolation of microglia from 'Young Donor' mice, and injection into recipient mouse brain. Recipient mice will consist of 84 total GFAP-Tau^{WT}/WT mice. (14 mice per day). [Same mice from Major Task 2].	Y2Q4	In progress, 50%
Subtask 6: Tissue harvest of inoculated 'Recipient' naïve mice, and PLX3397 treated mice, collection of brain tissue, sectioning, histopathological staining and analyses with astroglia, tau and other relevant antibodies. (N=72 mice).	Y3Q1 to Y3Q3	Not started
Subtask 7: LCD of microglia from mice in Major Task 1 (subtask 3), for single cell array for gene expression profiling. There will be 24 mouse brains per genotype for analyses. (N=72 mouse brains total).	Y3Q1 to Y3Q3	Not started
Subtask 8: Data analysis, validation, interpretation and correlation studies.	Y3Q4	Not started

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.

Below Are The Tasks Initiated Or Completed During This Reporting Period:

Major Task 1: Delineating the influence of APOE genotype on pathological and biochemical outcomes 3 and 6 Mos after r-mTBI/sham in a GFAP-hTau^{WT} mouse model with astrocyte specific (non-mutant) hTau expression.

This study requires 6 different transgenic lines –
APOE2-TR, APOE3-TR, APOE4-TR, hAPOE2*GFAP-Tau, hAPOE3*GFAP^{Tau} and hAPOE4*GFAP^{Tau} mice

All single APOE-TR lines have been generated and are due for their scheduled injuries in the next quarter. The hAPOE2*GFAP-Tau, hAPOE3* GFAP-Tau and hAPOE4*GFAP-Tau lines required two steps of breeding and are final genotype is currently being generated; 50% of the colonies are expected in the next two quarters. Mice will begin their injuries over the next two quarter periods.

Of note - Our breeding plan was affected (~4-5months) by the slowdown of activities in 2021 due to COVID-19 measures and a reduced workforce. We also encountered issues to do with pathogen infection from the Tau transgenic mice obtained from our collaborator. To prevent contamination with other mice in our vivarium we had to utilize mice rederivation services to generate pathogen free mice which took a period of 4 months. Altogether this set our breeding plan back by up to 9 months. As a result, we do not have any data to present on the mouse cohorts for this current year. However we plan to generate first set of data in the 2nd or 3rd quarters of the 2nd year of the project. We anticipate that we will require an additional 9 months of no-cost extension to complete this project due to the set-backs.

MAJOR TASK 2: We will delineate the contribution of astrocytes to the spreading of Tau proteinopathies, and subsequent neurodegeneration post-TBI, and how this is influenced by APOE genotype.

We began optimizing conditions for LCM and astroglial gene array analyses of human TBI/ADRD astrocytes. Work is being conducted by our collaborator (Dr Mufson) at Barrow Neurological Institute and he is planning to generate datasets within the next quarter.

We are also breeding cohorts for young donor mice APOE2, APOE3, APOE4-TRs and recipient GFAP-Tau mice for the cell inoculation studies along with mice from Major task 1.

MAJOR TASK 3: We will delineate the contribution of microglia in driving TBI-induced Tau astrogliopathies, and how this is influenced by APOE genotype.

We began optimizing conditions for LCM and microglial gene array analyses of human TBI/ADRD. As above, this work is being conducted by our collaborator (Dr Mufson) at Barrow Neurological Institute and he is planning to generate datasets within the next 4 months.

No final dataset has been generated 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.

OVER THE NEXT QUARTER WE PLAN TO CONTINUE OR BEGIN:
Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

1) Continue breeding of cohorts for injuries (24 mice per genotype hAPOE2, hAPOE3, hAPOE4, hAPOE2*GFAP-Tau^{WT}, hAPOE3* GFAP-Tau^{WT}, hAPOE4*GFAP-Tau^{WT}) at 3 & 6 months post-TBI Also include additional cohorts for microglia depopulation study.

2) Begin administering injuries or sham injuries at 3 months of age. Animals will receive 2 closed head injuries each week for 3 months, using a CCI device. There are 6 genotypes, 12 cr-mTBI and 12 cr-sham mice per group. A total of 144 mice will be used (72 cr-mTBI and 72 mice r-sham).

3) Continue breeding of cohorts for Young Donor mice for TBI studies in Major task 2 - Subtask 4 and 6 (12 mice per genotype for hAPOE2*GFAP-Tau, hAPOE3*GFAP-Tau , hAPOE4*GFAP-Tau).

4) Continue LCM studies of astrocytes and microglia in human tissue.

4. **IMPACT:** D*escribe 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 PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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.

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.

As mentioned above, due to COVID-19 measures in 2021, there was a significant reduction in work output. This set us back by around 4-5 months. Additionally, we also received a set back from our breeding schedule as the breeding pairs we requested from our collaborator did not pass the required pathogen test for our vivarium. We therefore had to rederive the mice from a pathogen free female from Jax's Lab. This also set us back by 4 months in our breeding schedule. Despite these set-backs, work is now progressing as normal and we aim to gain grounds over the next few quarters. Our breeding is now on full capacity, and injuries to animals has begun systematically. In light of these set-backs, we do anticipate that we will require a no-cost extension period of approx. 9 months to complete our mouse in vivo study.

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.

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

N/A

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

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. Describe the technologies or techniques were 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. 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;*
- *physical 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.*

Nothing to report

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".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: *Fiona Crawford (no change)*
Project Role: *PI*
Researcher Identifier (e.g. ORCID ID): *N/A*
Nearest person month worked: *1.2*
Contribution to Project: *Dr Crawford directs all aspects of this project and provides supervision on the overall approach and data interpretation for the experiments outlined in this application. In particular, she will interact with all of the team members listed on this application and provide full oversight as they implement the work proposed in this application, including regular communication with the expert consultants.*

Name: *Joseph Ojo (no change)*
Project Role: *Co-PI*
Researcher Identifier (e.g. ORCID ID): *N/A*
Nearest person month worked: *2.4*
Contribution to Project: *Dr. Ojo works alongside Dr. Crawford in directing all aspects of this project and providing supervision on mTBI animal modeling, histopathological analyses and data interpretation. He is responsible for overseeing all aspects of animal manipulation and ensuring that the projects are executed in a timely fashion. He will also perform histopathological assessments in both humans and animal models as described in the proposal.*

Name: *Camila Ortiz (no change)*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *N/A*
Nearest person month worked: *12*
Contribution to Project: *Ms. Ortiz will be involved in animal handling and surgical procedures, as well as histopathological characterization and molecular analyses, such as western blotting and ELISA. She will assist in all surgical procedures (primarily years 1 and 2) and conduct histopathological analyses with the supervision of Dr. Ojo (primarily Years 2 & 3).*

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.

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."

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);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Organization Name: Barrow Neurological Institute
Location of Organization: (if foreign location list country): Arizona, US
Partner's contribution to the project: Collaboration (subaward site) – Dr Mufson uses his laser capture microdissection technique to capture astrocytes and microglia on autopsy tissue of TBI/ADRD cases, and conducts single cell array for novel TBI induced astrocyte/microglia gene profiles. This work is currently ongoing and planned completion will be next year.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (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.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

Please find the quad chart attached on the next page.

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.*

Please find the quad chart attached on the next page.

Impact of APOE Genotype on Astrogliopathies in Repetitive mTBI

Log Number AZ190028

W81XWH-20-1-0281

PI's: Drs Fiona Crawford / Joseph Ojo **Org:** Roskamp Institute, Sarasota, FL **Award Amount: $747K**

Study Aim(s)

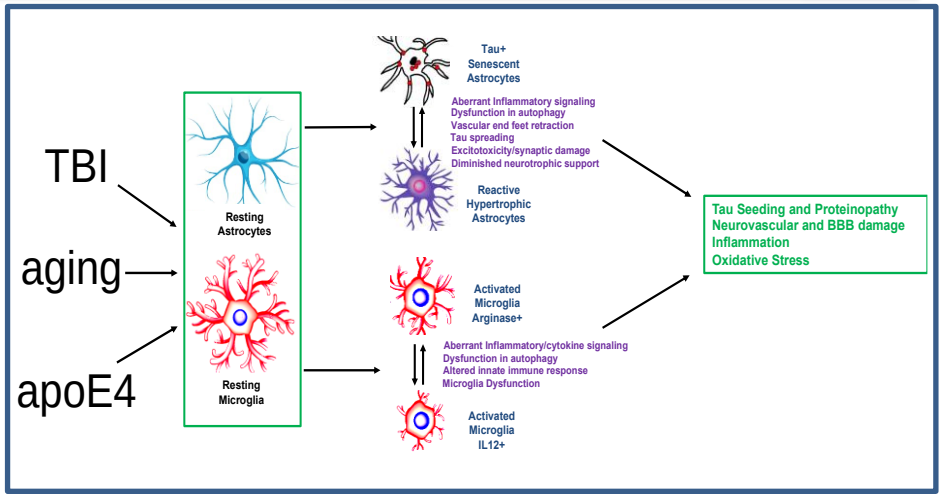
AIM 1: Delineating the influence of APOE genotype on pathological and biochemical outcomes 3 and 6 Mos after r-mTBI/sham in a GFAP-hTau mouse model with astrocyte specific (non-mutant) hTau expression.
AIM 2: To delineate the contribution of astrocytes to the spreading of Tau proteinopathies, and subsequent neurodegeneration post-TBI, and how this is influenced by APOE genotype.
AIM 3: To delineate the contribution of microglia in driving TBI-induced Tau astrogliopathies, and how this is influenced by APOE genotype.

Approach

i) Histopathology of astrocyte pathobiology in APOE-GFAP/Tau mouse models exposed to r-mTBI/sham injury at 3 mos and analyzed at 3 & 6mos post-injury.

ii) Adoptive transfer of TBI astrocytes into naïve mice (in vivo), inoculation of APOE-TR mice with astrocyte derived Tau, exposure of TBI astrocyte secretomes to healthy neural/vascular cells (ex vivo). Assessment of pathobiological changes induced by reactive astrocytes. Single cell profiles of astrocytes from autopsy and mouse models.

iii) Adoptive transfer of TBI microglia into naïve mice (in vivo), ablation of microglia in APOE-TR/GFAP-Tau sham/r-mTBI mice. Assessment of pathobiological changes induced by reactive microglia. Single cell profiles of microglia from autopsy and mouse models.



Accomplishment: Continued breeding of APOE-TR and APOE-TR/GFAP-tau mice for planned studies. Began pilot astrocyte LCM/gene array studies for optimizing conditions for the experiments.

Timeline and Cost

Activities CY	20-21	21-22	22-23
MAJOR TASK ONE OR AIM 1			
MAJOR TASK TWO OR AIM 2			
MAJOR TASK TWO OR AIM 3			
Estimated Direct Budget (500K)	**$90K**	**$194K**	**$200K**

Last updated: (September 2020)

Goals/Milestones

CY20 Goal
☑ Obtain regulatory approval to begin animal and human specimen studies
CY21 Goals
☑ Breeding and administering injuries to different mouse models
☑ Begin gene analyses of microdissected astrocyte from autopsy tissue
CY22 Goals
☑ Histological assessment of astrocytes and proteinopathy in mouse models
☑ Conduct adoptive transfer studies, ex vivo functional assay studies and astrocyte derived tau inoculation studies.
☑ Complete human gene array analyses and astrocyte gene analyses
CY23 Goal
☑ Histological assessment of astrocytes and proteinopathy in mouse models
☑ Conduct adoptive transfer studies and microglia ablation studies.
☑ Complete human gene array analyses and microglia gene analyses
Comments/Challenges/Issues/Concerns
- **N/A**

Projected Expenditure: $145,312 **Actual Expenditure**: $199,010.73.