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TITLE: **Impact of APOE Genotype on Astrogliopathies in Repetitive mTBI**

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Impact of APOE Genotype on Astrogliopathies in Repetitive mTBI

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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**
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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, 100%
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	In progress, 70%
Subtask 4: Tissue harvest of the 3 months cohort from subtask 3.	Y2Q1	In progress, 25%
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	Completed 100%

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, 100%
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	In progress, 100%
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	In progress, 50%
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	In progress, 50%
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	In progress, 10%
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	In progress, 100%
Subtask 2: Breeding of cohorts for Donor mice in subtask 5.	Y1Q4	In progress, 50%
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	In progress, 10%
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 the final genotype currently being generated; colonies have now been generated. Mice are currently undergoing their injuries, and we anticipate that these should be completed over the next quarter.

Of note – As indicated in our previous reports, our breeding plan was affected (~6months) 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 6 months. Altogether this set our breeding plan back by over 12 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 quarter of the 3rd year of the project. We anticipate that we will require an additional 12 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 completed LCM and astroglial gene array analyses of human TBI/ADRD astrocytes. Work was conducted by our collaborator (Dr Mufson) at Barrow Neurological Institute. We have generated some preliminary data for presentation in this report, but plan a more extensive bioinformatic analyses in the next coming weeks for future report presentation.

We have almost completed breeding cohorts for recipient mice in the cell inoculation studies along with mice from Major task 1. These experiments begin in the week beginning 17th Oct 2022.

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 are breeding cohorts for mice planned for the PLX depletion study in Major task 3. These experiments will begin in the next quarter.

We also completed LCM and microglial gene array analyses of human TBI/ADRD. As above, we have generated some preliminary data for presentation in this report, but plan a more extensive bioinformatic analyses in the next coming weeks for future report presentation.

Gene array data from human brains (hippocampus) – microglial and astroglia LCM analyses.
N=6 Non demented controls
N=13 TBI APOE4 carriers
N=8 TBI APOE/E3 carriers
Demographic and clinical information pending.

METHODOLOGY

LASER CAPTURE MICRODISSECTION (LCM)AND GENE TRANSCRIPT PROFILING:

A laser capture microdissection (LCM) instrument (Mufson lab) was used for these studies. LCM methodology results in accurate dissection of the microglial cells of interest with minimal disruption of the surrounding tissue. For the proposed studies, IBA1-positive microglia/resident macrophages and GFAP+ astrocytes are captured per reaction, per condition for subsequent linear terminal continuation (TC) RNA amplification and microarray analysis. TC RNA amplification was developed in Dr. Ginsberg's laboratory. The method entails synthesizing first strand cDNA complementary to the RNA template, generating second strand cDNA complementary to the first strand cDNA, and finally *in vitro* transcription using the double stranded cDNA as template. This process displays high fidelity, reproducibility and increased signal sensitivity (4-fold) and flexibility compared to other RNA amplification protocols. Radiolabeled RNA probes will be hybridized to custom-designed microarrays. Array platforms consist of 1μg of linearized cDNA purified from plasmid preparations adhered to high-density nitrocellulose (Hybond XL, GE Healthcare, Piscataway, NJ) using an arrayer robot (VersArray, Bio-Rad, Hercules, CA) (Ginsberg, 2005, 2008). Each cDNA and/or expressed sequence-tagged cDNA (EST) was verified by sequence analysis and restriction digestion. Human clones are employed on the custom-designed array. Approximately 864 cDNAs/ESTs organized into 22gene ontology groups are present on the current array platform. Dr. Ginsberg has already successfully and productively transferred this technology to Dr. Mufson's laboratory at Barrow Neurological Institute, which is where these experiments were carried out. **Statistical procedures** (using GeneLinker Gold**)** for custom-designed microarray analysis have been described in detail previously (Ginsberg et al., 2014; 2016; 2017; Mufson et al., 2017). Gene expression differences will be assessed with respect to the hybridization signal intensity ratio of the total signal of all array genes. For each gene the signal intensity ratio is modelled as a function of study group, using mixed effects models with random effect to account for the correlation between repeated assays. Between subject versus within-subject variation will be analyzed by random intercept, fixed effect covariate, Kenward-Roger denominator degrees of freedom, unstructured covariance structure, and log-transformed expression levels. Significance will be judged at the level $\alpha=0.01$, two-sided; false discovery rate (FDR) based on an empirical null distribution due to strong correlation between genes is controlled at level $\alpha=0.1$. Spearman's coefficient will evaluate correlations of select transcripts. Serial section *in situ* hybridization will confirm changes in transcript expression.

Microglia

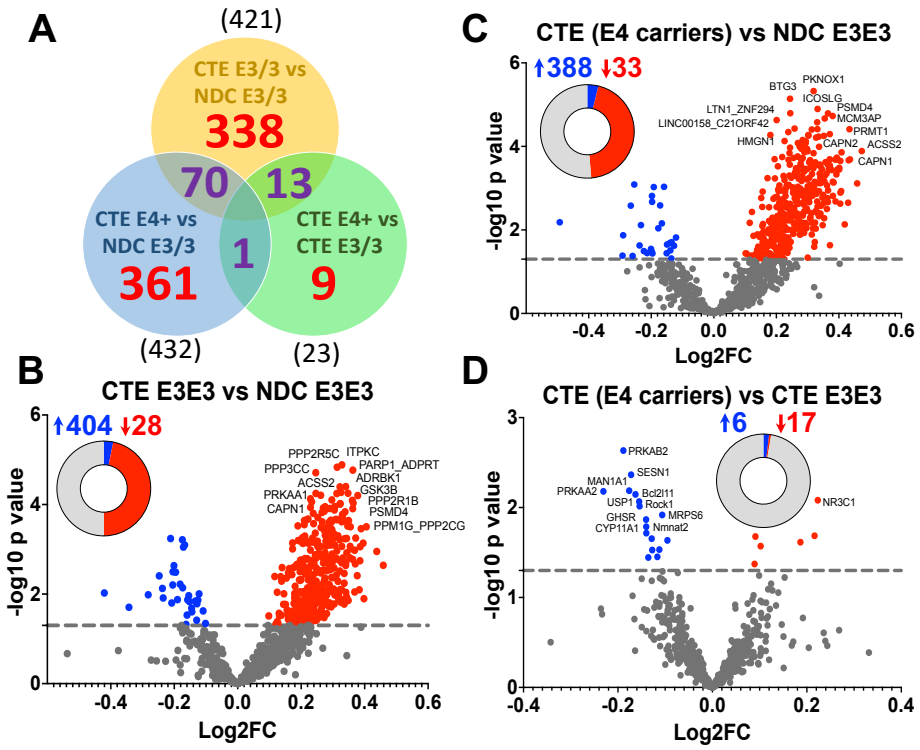


Fig 1: Differentially expressed genes (DEGs) and Volcano plot of gene array data obtained from IBA1+ micro-dissected cells from the hippocampus of TBI (APOE3/E3 and APOE4 carriers) vs non demented controls (NDC) APOE3/E3 subjects. (A) Venn diagram of DEGs. **(B-D) Volcano plots** of DEGs from TBI APOE3/E3 vs NDC APOE3/E3 (B), TBI APOE4 carriers vs NDC APOE3/E3 (C), and TBI APOE4 carriers vs TBI APOE3/E3 groups (D).

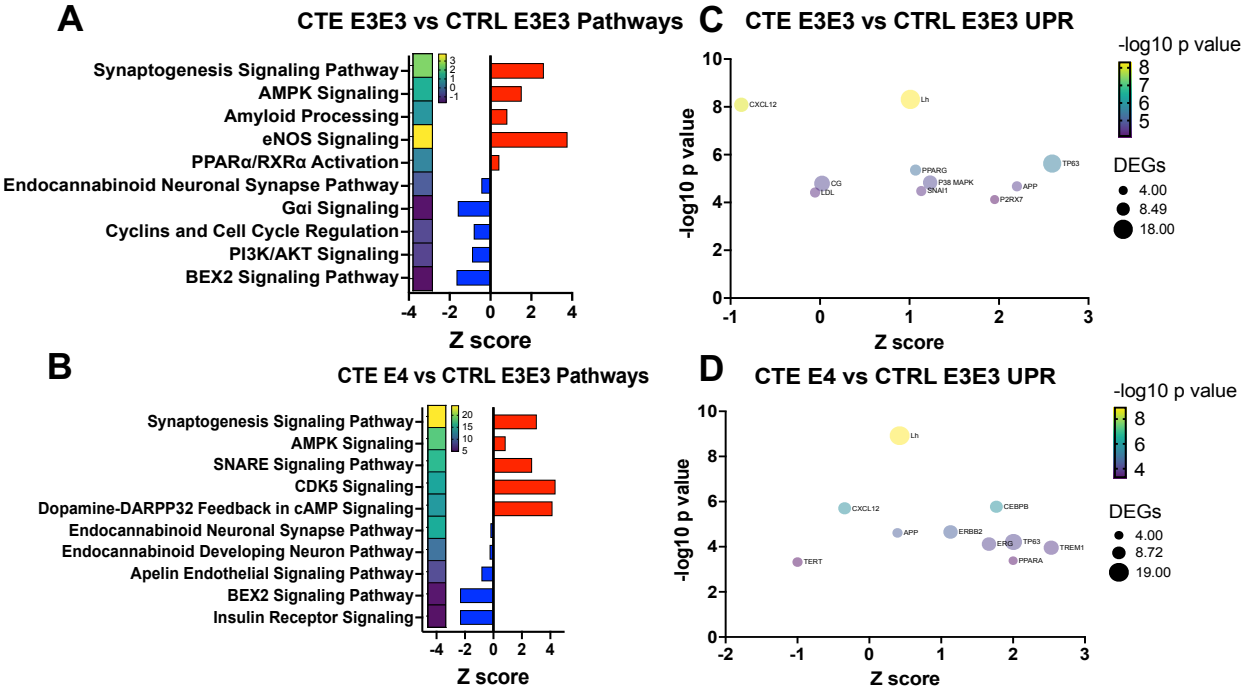


Fig 2: Canonical pathways and upstream regulators identified in IBA1 microdissected cells from the brains of TBI (APOE3/E3 and APOE4 carriers) vs non demented controls (NDC) APOE3/E3 subjects. Top 5 up- and down-regulated canonical pathways in IBA1+ brain resident cells altered between TBI APOE3/E3 vs NDC APOE3/E3 **(A)** and TBI APOE4 carriers vs NDC APOE3/E3 groups **(B).** Corresponding Top 10 upstream regulators are shown in the bubble plots in **C** and **D,** respectively.

Summary of findings on microglia (discussion)

1) We observed 421 VS 432 DEGs that were altered between TBI APOE3/3 vs non demented control (NDC) APOE3/3 and TBI APOE4 carrier vs NDC APOE3/3 subjects, respectively.
2) Most significantly altered genes were unique to both comparisons above, only 70 DEGs were found to overlap.
3) Some dysregulated pathways identified by ingenuity pathway analysis were similar between both comparisons above (e.g. AMPK, endocannabinoid and BEX2 signaling). There were notable differences such as amyloid processing, eNOS PI3K/AKT in the TBI APOE3/3 vs non demented control (NDC) APOE3/3 comparison, and CDK5, Dopamine, Apelin and Insulin receptor signaling in the TBI APOE4 carrier vs NDC APOE3/3 comparison.
4) Very minimal changes were observed when we compared TBI APOE4 carrier vs TBI APOE3/3 cases, only 23 DEGs were identified.

Astroglia

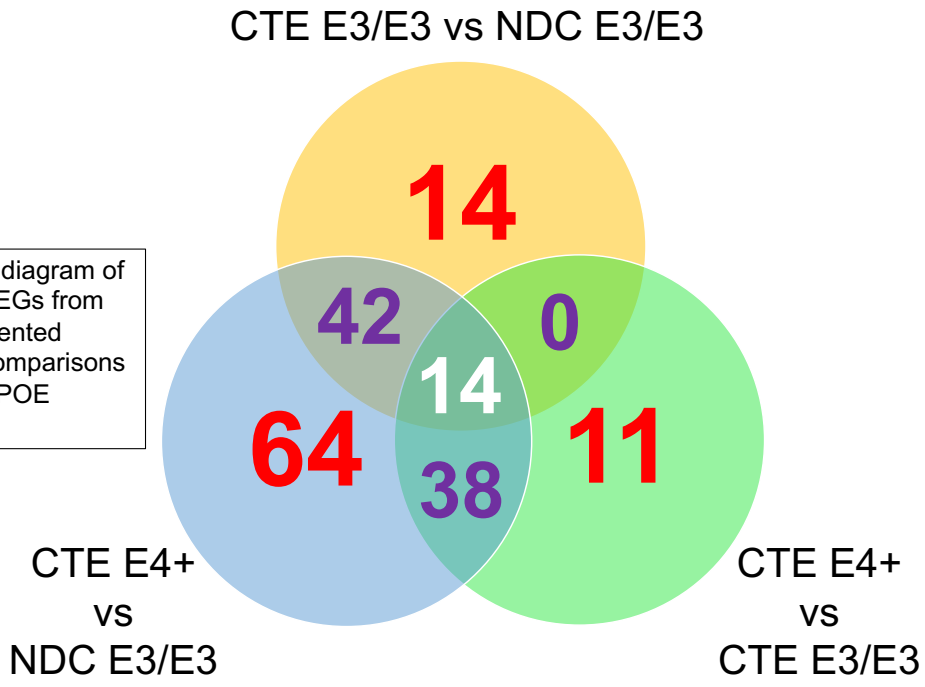


Figure 3: Venn diagram of the astrocyte DEGs from cases/non-demented control group comparisons from different APOE carriers.

Table 1

CTE E3E3 vs NDC E3E3			
Gene ID	Gene ontology	P value	LogFC
ARHGDIB	EALS	0.0007	-0.1259
STMN2	NT	0.0008	-0.1561
STMN1	NT	0.0011	-0.1490
M6PR/CDMPR	EALS	0.0011	-0.1279
PTTG1IP	DS	0.0020	-0.1146
TMPRSS15/PRSS7	DS	0.0022	-0.1926
CCK	PEP	0.0037	-0.1669
CHRNA7	MONO	0.0043	-0.2127
CNR1	PEP	0.0045	-0.1782
NTRK3TK	NT	0.0045	-0.1466
SLC6A2/NET	MONO	0.0047	-0.1617
CHRNA4	MONO	0.0052	-0.1540
TMEM50B/C21ORF4	DS	0.0057	-0.1646
FMR1	DV	0.0058	-0.1609
CHRNA3	MONO	0.0062	-0.1650
ATG3	EALS	0.0069	-0.1262
FKTN/FCMD	DV	0.0071	-0.1577
TSC2	DV	0.0072	-0.1256
FOS	IE	0.0074	-0.1613
MAPT1	AD	0.0076	-0.2040
TH	MONO	0.0084	-0.1346
SLC18A3/VACHT	MONO	0.0086	-0.1364
GAD1/GAD67	GABA	0.0094	-0.2165
PAM	MONO	0.0096	-0.1446
NTRK3ECD	NT	0.0098	-0.1347
TFF1	DS	0.0099	-0.0885
BDNF	NT	0.0105	-0.1412
PSMG1/DSCR2	DS	0.0109	-0.1046
PRMT2	DS	0.0110	-0.0698
RGS9	GP	0.0113	-0.1461
LRP1	AD	0.0114	-0.1984
CHRNB2	MONO	0.0114	-0.1595
NTRK2TK	NT	0.0126	-0.1282
ATG4A	EALS	0.0135	-0.1133
RGS4	GP	0.0135	-0.1507
FOSL2/FOSLR	IE	0.0136	-0.1639
RGS3	GP	0.0158	-0.1458
EEA1	EALS	0.0192	-0.0880
TFF2	DS	0.0209	-0.1302
PWP2	DS	0.0230	-0.0912

CTE E3E3 vs NDC E3E3			
Gene ID	Gene ontology	P value	LogFC
FOSB	IE	0.0230	-0.1422
MX1	DS	0.0233	-0.1387
MRPS6	DS	0.0234	-0.1302
KCNJ15	DS	0.0240	-0.1177
RRP1B	DS	0.0247	-0.1445
JUN/CJUN	IE	0.0250	-0.1509
EMX2	DV	0.0266	-0.1143
NRP1/NRP	NT	0.0273	-0.1270
MAOB	MONO	0.0275	-0.1192
GRM4	GLUR	0.0297	-0.1151
CHRNB1	MONO	0.0302	-0.1543
RAB5A	EALS	0.0307	-0.1509
DCX	DV	0.0313	-0.3156
ZBTB21/ZNF295	DS	0.0320	-0.0830
SLC6A4/SERT	MONO	0.0326	-0.1740
PPT1	EALS	0.0331	-0.1329
MRAP	DS	0.0335	-0.1081
TCP10L	DS	0.0337	-0.0863
HTR3A/HTR3	MONO	0.0359	-0.1121
CHODL	DS	0.0366	-0.1146
KCNIP3	AD	0.0376	-0.1685
DSCR9	DS	0.0379	-0.1367
SLC6A12/GAT2	GABA	0.0401	-0.1339
DSCR8	DS	0.0427	-0.1234
FLNA	CYT	0.0441	-0.1262
ATG4B	EALS	0.0446	-0.4502
ATG4D	EALS	0.0475	-0.1439
RAB9	EALS	0.0481	-0.1139
TSC1	DV	0.0484	-0.0979
CHRM2	MONO	0.0491	-0.1176

Table 2

CTE E 4+ vs NDC E3/E3			
Gene ID	Gene ontology	P value	LogFC
TMPRSS15/PRSS7	DS	0.0000	-0.3159
TMEM50B/C21ORF4	DS	0.0000	-0.2787
RIPPLY3/DSCR6	DS	0.0000	-0.2614
FKTN/FCMD	DV	0.0001	-0.2404
KCNJ6	DS	0.0001	-0.3160
FAS/FASLR	CD	0.0001	-0.3127
KCNE2	DS	0.0001	-0.2192
RBM11	DS	0.0001	-0.3202
FMR1	DV	0.0001	-0.2367
MRPS6	DS	0.0001	-0.2485
MRAP	DS	0.0001	-0.2356
DSCR8	DS	0.0001	-0.2694
ATG4D	EALS	0.0001	-0.2582
ATG7	EALS	0.0002	-0.2479
MAPT1	AD	0.0002	-0.2879
KCNB1	CH	0.0002	-0.2554
DSCR9	DS	0.0002	-0.3056
BAD	CD	0.0002	-0.2618
NEFM/NEF3	CYT	0.0003	-0.0921
KRAS/RAS	CD	0.0003	-0.2948
CNR1	PEP	0.0003	-0.2470
DAXX	CD	0.0003	-0.2761
VCAN/CSPG2	CYT	0.0004	-0.2625
KCNJ15	DS	0.0004	-0.2209
CHAF1B	DS	0.0005	-0.2521
TFF2	DS	0.0005	-0.2450
HTT/HD	AD	0.0005	-0.2816
KCNE1	DS	0.0006	-0.2482
ABCC13	DS	0.0006	-0.2592
KCNIP3	AD	0.0007	-0.2694
PTTG1IP	DS	0.0008	-0.1930
TFF1	DS	0.0009	-0.1972
PWP2	DS	0.0009	-0.2017
TCP10L	DS	0.0011	-0.1635
DIABLO/SMAC	CD	0.0012	-0.3606
CAV1	CYT	0.0012	-0.1372
CCT8	DS	0.0013	-0.2100
AIFM1/PCD8	CD	0.0013	-0.1811
TH	MONO	0.0014	-0.1981
STMN2	NT	0.0016	-0.1737

CTE E 4+ vs NDC E3/E3			
Gene ID	Gene ontology	P value	LogFC
ADAMTS1	DS	0.0016	-0.2379
BDNF	NT	0.0017	-0.2066
ATG5	EALS	0.0018	-0.1973
BAK1	CD	0.0018	-0.2531
CCK	PEP	0.0019	-0.2199
HDLBP	AD	0.0020	-0.2298
FOS	IE	0.0021	-0.2086
BACE2	AD	0.0022	-0.2867
PSMG1/DSCR2	DS	0.0025	-0.2201
CHRNA7	MONO	0.0031	-0.3046
MEMO1P1/C21ORF19	DS	0.0031	-0.2565
LRP1	AD	0.0032	-0.2465
SLC6A13/GAT3	GABA	0.0033	-0.2268
CHRNB2	MONO	0.0033	-0.2107
DSCR4	DS	0.0034	-0.1772
JAM2	DS	0.0036	-0.2021
CHRNB3	MONO	0.0036	-0.2164
ARHGDIB	EALS	0.0037	-0.1481
GNAL	GP	0.0038	-0.1954
CBS	DS	0.0041	-0.1825
CHODL	DS	0.0043	-0.2253
BCL2	CD	0.0043	-0.2363
DPP6	CH	0.0043	-0.1026
JUN/CJUN	IE	0.0044	-0.2246
FLNA	CYT	0.0049	-0.2075
CCNF	CD	0.0050	-0.2138
ADAMTS5	DS	0.0052	-0.2313
GRM6	GLUR	0.0053	-0.2103
WRB	DS	0.0055	-0.2102
FOSL2/FOSLR	IE	0.0058	-0.1959
RGS9	GP	0.0062	-0.1640
BACE1	AD	0.0063	-0.2750
ZBTB21/ZNF295	DS	0.0065	-0.1159
BPTF/FALZ	AD	0.0066	-0.2066
STMN1	NT	0.0066	-0.1696
SAA4	AD	0.0068	-0.2050
DPP10	CH	0.0068	-0.1314
BAX	CD	0.0071	-0.2186
PRMT2	DS	0.0071	-0.1675
EPYC/DSPG3	CYT	0.0083	-0.1704

CTE E 4+ vs NDC E3/E3			
Gene ID	Gene ontology	P value	LogFC
PAXBP1/C21ORF66	DS	0.0083	0.3916
GNAQ	GP	0.0085	-0.1975
MORC3/ZCWCC3	DS	0.0086	-0.1725
ATG4A	EALS	0.0092	-0.1337
RAB3A	EALS	0.0098	0.3398
FADD	CD	0.0099	-0.4022
VLDLR	AD	0.0107	-0.2113
FOSB	IE	0.0108	-0.1715
C2CD2/C21ORF25	DS	0.0114	-0.1628
CHRNB1	MONO	0.0116	-0.2115
ITGB2	DS	0.0121	-0.1874
CCNG1	CD	0.0124	-0.1815
MCM3AP	DS	0.0127	-0.1617
NRIP1	DS	0.0135	0.3641
RAB4A	EALS	0.0144	0.2390
GABBR1	GABA	0.0150	0.3383
ITSN1	DS	0.0151	-0.2986
CLDN17	DS	0.0157	0.4270
SLC6A2/NET	MONO	0.0161	-0.2158
SLC18A3/VACHT	MONO	0.0169	-0.1553
CCND2	CD	0.0175	-0.2053
TSC2	DV	0.0190	-0.1366
NTRK3TK	NT	0.0195	-0.1549
PSENEN	AD	0.0209	-0.1994
NRP1/NRP	NT	0.0213	-0.1673
CXADR	DS	0.0220	0.3689
M6PR/CDMPR	EALS	0.0223	-0.1063
DYNLL1/DNCLC1	CYT	0.0223	-0.1534
HMGCS1	PROT	0.0225	0.6567
RAB6A	EALS	0.0228	0.3548
PRDM15	DS	0.0236	-0.3920
NFKB1/NFKB	CD	0.0244	-0.2442
MAP3K7CL/C21ORF7	DS	0.0247	-0.1672
SLC6A12/GAT2	GABA	0.0247	-0.1548
ASTN2	CYT	0.0251	-0.1280
CLIC6	CH	0.0260	-0.2672
CHMR4	MONO	0.0262	-0.2650
IGSF5	DS	0.0275	-0.1559
DISC1	DV	0.0278	-0.1361
EMX2	DV	0.0280	-0.1215

CTE E 4+ vs NDC E3/E3			
Gene ID	Gene ontology	P value	LogFC
CHRNA3	MONO	0.0295	-0.2041
CTSB	EALS	0.0300	0.2653
NRG2	DV	0.0313	-0.3731
SUMO3	DS	0.0316	-0.5295
SLC2A1	GLUC	0.0323	-0.1846
DCX	DV	0.0326	-0.4233
SORT1	EALS	0.0329	-0.3322
TPTE	DS	0.0331	0.2593
CHRNA4	MONO	0.0331	-0.1793
MYO5A	EALS	0.0337	-0.2512
RGS4	GP	0.0338	-0.1747
ATG3	EALS	0.0338	-0.1188
GRM4	GLUR	0.0340	-0.1202
CCND1	CD	0.0360	-0.2000
NTRK3ECD	NT	0.0374	-0.1496
GRIK1	GLUR	0.0382	0.3735
GRIA2	GLUR	0.0384	0.3973
NTRK2TK	NT	0.0385	-0.1476
MSR1	GLIA	0.0396	0.5000
MYO5B	EALS	0.0404	-0.4038
CASP8	CD	0.0405	-0.3922
LTN1/ZNF294	DS	0.0407	-0.1570
DUSP5	PP/K	0.0428	0.2134
NPC1	EALS	0.0431	-0.5776
CLCN5	CH	0.0453	-0.1624
NEFL	CYT	0.0454	-0.4334
POTED/ANDRK21	CYT	0.0455	-0.0616
CLCN7	CH	0.0459	-0.0884
DRD4	MONO	0.0459	-0.2265
CTSG	EALS	0.0459	-0.2825
SLC6A11/GAT4	GABA	0.0462	-0.5205
SLC6A4/SERT	MONO	0.0462	-0.2214
CTSL	EALS	0.0462	-0.3434
C21ORF79	DS	0.0483	-0.1250
AQP1	CYT	0.0489	-0.4947
CCNE1	CD	0.0493	-0.1296
CTSE	EALS	0.0497	-0.4052
ADCY8	GP	0.0497	-0.3249

Table 3

CTE E4+ vs CTE E3E3			
Gene ID	Gene ontology	P value	LogFC
FAS/FASLR	CD	0.0055	-0.2582
RBM11	DS	0.0069	-0.1934
KRAS/RAS	CD	0.0075	-0.1972
KCNJ6	DS	0.0088	-0.1821
DSCR9	DS	0.0115	-0.1690
MEMO1P1/C21ORF19	DS	0.0118	-0.1556
TFF1	DS	0.0120	-0.1087
DSCR8	DS	0.0126	-0.1460
PSMG1/DSCR2	DS	0.0148	-0.1155
MCM3AP	DS	0.0149	-0.1178
RAB4A	EALS	0.0149	0.1938
DAXX	CD	0.0153	-0.1994
MRAP	DS	0.0157	-0.1275
VCAN/CSPG2	CYT	0.0160	-0.1670
PRDM15	DS	0.0160	-0.2335
PRMT2	DS	0.0162	-0.0978
CLIC6	CH	0.0173	-0.1747
ITSN1	DS	0.0174	-0.1462
CHAF1B	DS	0.0187	-0.1466
BAD	CD	0.0196	-0.1557
PWP2	DS	0.0207	-0.1105
MRPS6	DS	0.0210	-0.1183
KCNE2	DS	0.0216	-0.1278
ABCC13	DS	0.0221	-0.1697
PAXBP1/C21ORF66	DS	0.0236	0.2767
PTTG1IP	DS	0.0241	-0.0784
DIABLO/SMAC	CD	0.0247	-0.2866
FADD	CD	0.0259	-0.3006
CASP8	CD	0.0281	-0.2388
KCNB1	CH	0.0284	-0.1544
KCNE1	DS	0.0296	-0.1354
KCNJ15	DS	0.0298	-0.1032
DCX	DV	0.0299	-0.1077
RAB3A	EALS	0.0299	0.2596
RIPPLY3/DSCR6	DS	0.0300	-0.1770
PKNOX1	DS	0.0310	-0.2542
SLC19A1	DS	0.0314	0.1472
TFF2	DS	0.0324	-0.1148
MAP3K7CL/C21ORF7	DS	0.0334	-0.1143
CAV1	CYT	0.0337	-0.1103

CTE E4+ vs CTE E3E3			
Gene ID	Gene ontology	P value	LogFC
LTN1/ZNF294	DS	0.0345	-0.1199
CLCN4	CH	0.0347	-0.3552
JAM2	DS	0.0353	-0.1172
IGSF5	DS	0.0358	-0.0953
IFNAR1	DS	0.0365	-0.0908
CXADR	DS	0.0369	0.2482
IFNAR2	DS	0.0393	-0.1134
APPL1	EALS	0.0401	0.3217
CLCN2	CH	0.0404	-0.4478
PDXK	DS	0.0405	-0.1363
GRIA4	GLUR	0.0407	0.2335
CCT8	DS	0.0412	-0.1154
ADAMTS1	DS	0.0424	-0.1415
AQP1	CYT	0.0425	-0.3593
HTT/HD	AD	0.0427	-0.1411
ASTN2	CYT	0.0427	-0.1143
CHMR4	MONO	0.0444	-0.1404
TMPRSS15/PRSS7	DS	0.0446	-0.1233
POFUT2	DS	0.0473	-0.4712
TMEM50B/C21ORF4	DS	0.0474	-0.1140
OLIG1	DS	0.0487	-0.2639
GABBR1	GABA	0.0490	0.2405
ITGB2	DS	0.0494	-0.1138

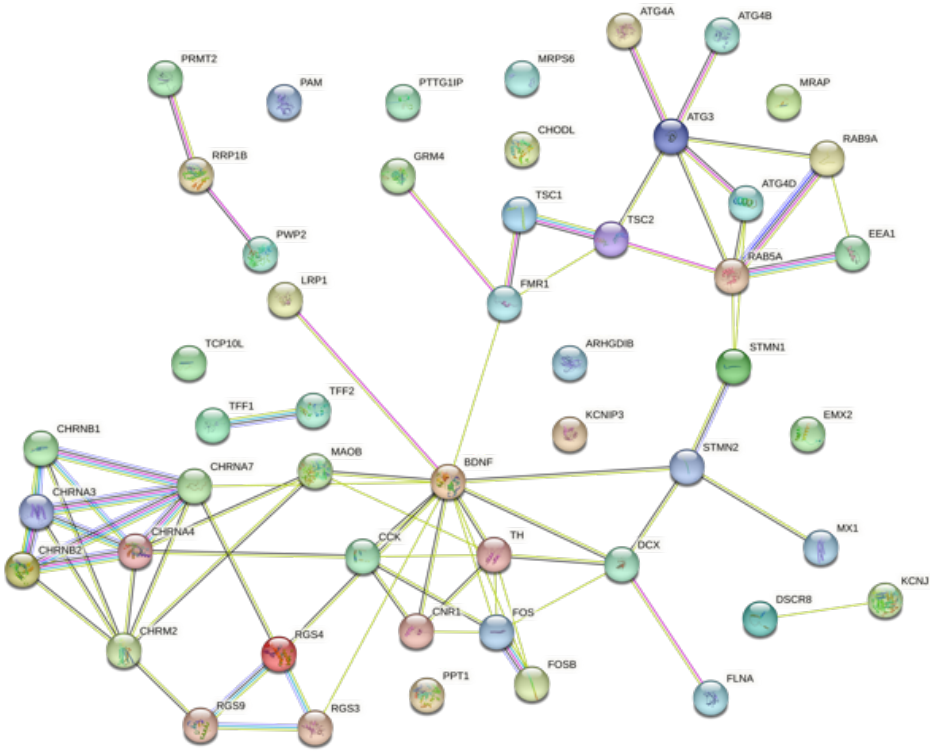


Figure 4: STRING protein-protein interaction network generated from DEG's between CTE E3E3 vs NDC E3E3

Table 4: Reactome pathways generated from DEG's between CTE E3E3 vs NDC E3E3

#term ID	term description	observed gene count	background gene count	strength	false discovery rate
HSA-629594	Highly calcium permeable postsynaptic nicotinic acetylcholine receptors	4	11	2.15	0.00011
HSA-1632852	Macroautophagy	6	133	1.25	0.00076
HSA-629587	Highly sodium permeable postsynaptic acetylcholine nicotinic receptors	3	7	2.22	0.00080
HSA-629597	Highly calcium permeable nicotinic acetylcholine receptors	3	9	2.12	0.0010
HSA-165181	Inhibition of TSC complex formation by PKB	2	3	2.42	0.0153
HSA-418594	G alpha (i) signalling events	7	396	0.84	0.0153
HSA-162582	Signal Transduction	18	2741	0.41	0.0173
HSA-112314	Neurotransmitter receptors and postsynaptic signal transmission	5	203	0.98	0.0332
HSA-8854214	TBC/RABGAPs	3	44	1.43	0.0390
HSA-9007101	Rab regulation of trafficking	4	120	1.12	0.0434

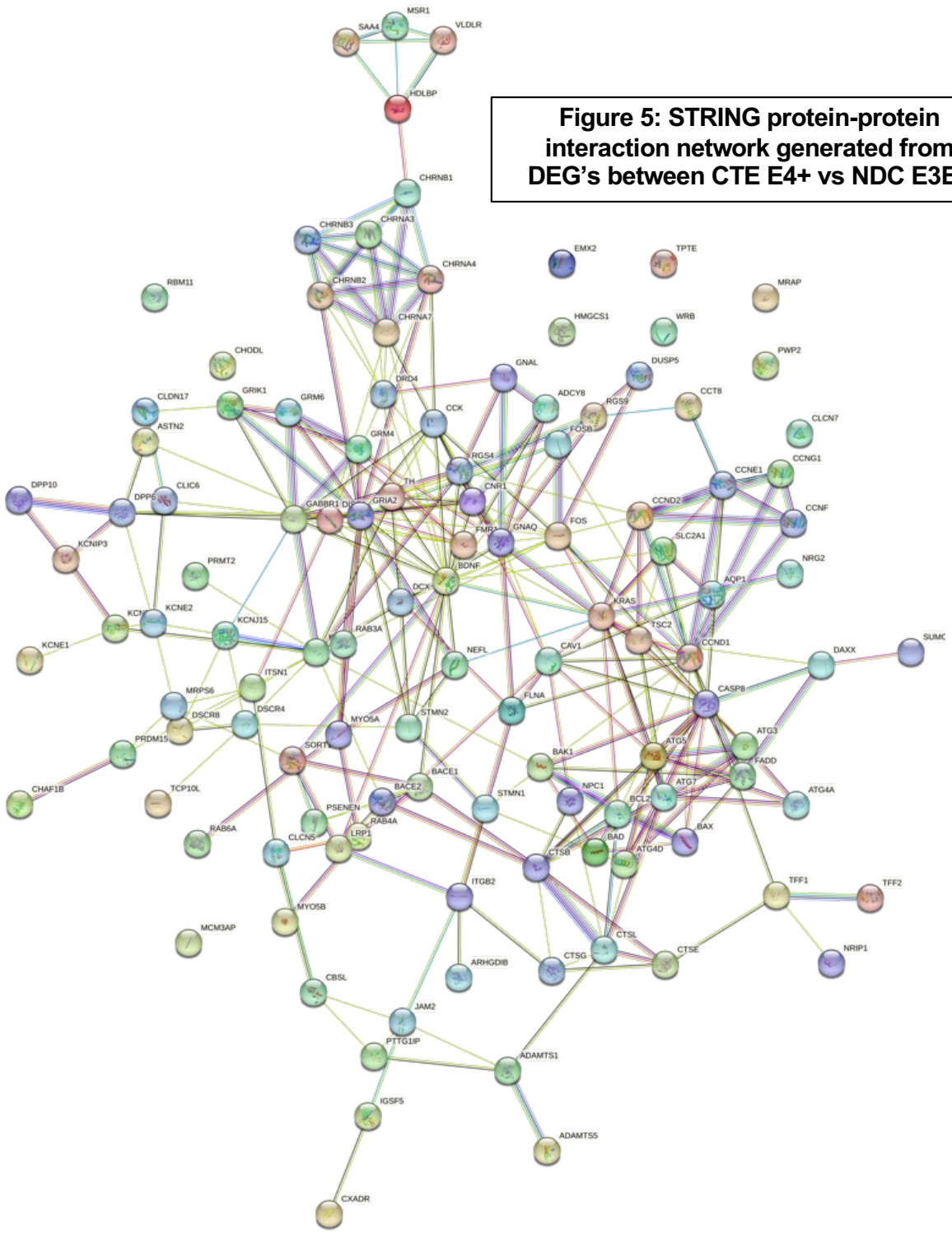


Figure 5: STRING protein-protein interaction network generated from DEG's between CTE E4+ vs NDC E3E3

Table 5: Reactome pathways generated from DEG's between CTE E4+ vs NDC E3E3

#term ID	Term description	Observed gene count	background gene count	strength	false discovery rate
HSA-112314	Neurotransmitter receptors and postsynaptic signal transmission	14	203	1.06	9.45E-08
HSA-112315	Transmission across Chemical Synapses	15	267	0.97	1.41E-07
HSA-112316	Neuronal System	16	406	0.82	3.03E-06
HSA-629594	Highly calcium permeable postsynaptic nicotinic acetylcholine receptors	5	11	1.88	1.53E-05
HSA-629597	Highly calcium permeable nicotinic acetylcholine receptors	4	9	1.87	0.00025
HSA-162582	Signal Transduction	37	2741	0.35	0.00027
HSA-991365	Activation of GABAB receptors	5	43	1.29	0.0021
HSA-109606	Intrinsic Pathway for Apoptosis	5	51	1.21	0.0037
HSA-629587	Highly sodium permeable postsynaptic acetylcholine nicotinic receptors	3	7	1.86	0.0039
HSA-8939211	ESR-mediated signaling	8	189	0.85	0.0039
HSA-9009391	Extra-nuclear estrogen signaling	5	74	1.05	0.0138
HSA-9018519	Estrogen-dependent gene expression	6	119	0.93	0.0138
HSA-9675126	Diseases of mitotic cell cycle	4	37	1.26	0.0138
HSA-418594	G alpha (i) signalling events	10	396	0.63	0.0179
HSA-1632852	Macroautophagy	6	133	0.88	0.0199
HSA-9661069	Defective binding of RB1 mutants to E2F1,(E2F2, E2F3)	3	17	1.47	0.0224
HSA-168898	Toll-like Receptor Cascades	6	152	0.82	0.0317
HSA-8848021	Signaling by PTK6	4	53	1.1	0.0317
HSA-1236394	Signaling by ERBB4	4	58	1.06	0.0393
HSA-9634638	Estrogen-dependent nuclear events downstream of ESR-membrane signaling	3	24	1.32	0.0404

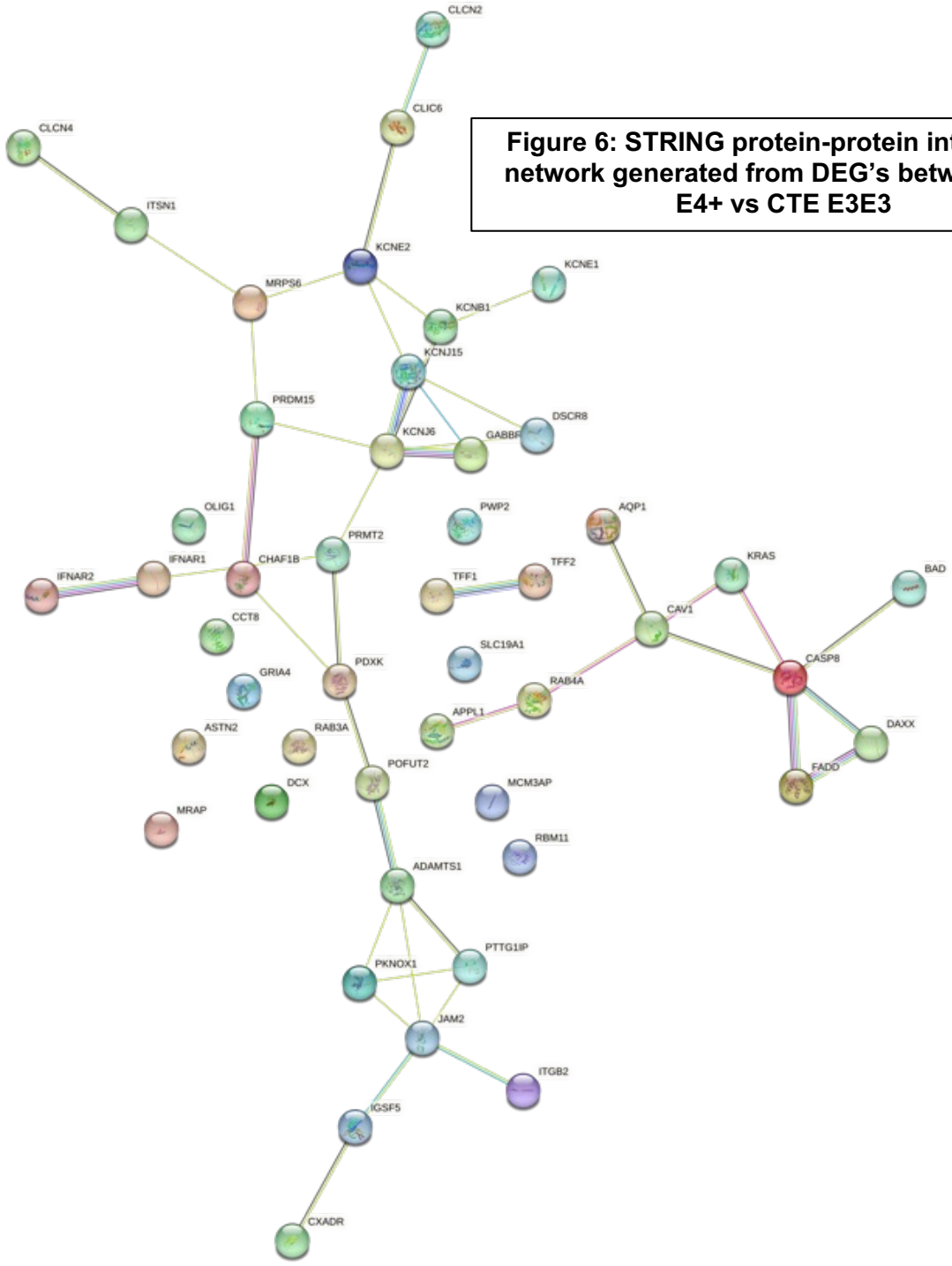


Figure 6: STRING protein-protein interaction network generated from DEG's between CTE E4+ vs CTE E3E3

Table 6: Reactome pathways generated from DEG's between CTE E4+ vs CTE E3E3

#term ID	term description	observed gene count	background gene count	strength	false discovery rate
GO:0022836	Gated channel activity	10	332	1.09	2.68E-05
GO:0005244	Voltage-gated ion channel activity	8	194	1.23	4.73E-05
GO:0005216	Ion channel activity	10	425	0.98	6.47E-05
GO:0005267	Potassium channel activity	6	123	1.3	0.00033
GO:0005249	Voltage-gated potassium channel activity	5	86	1.37	0.0011
GO:0015318	Inorganic molecular entity transmembrane transporter activity	11	813	0.74	0.0012
GO:0015075	Ion transmembrane transporter activity	11	865	0.71	0.0018
GO:0099094	Ligand-gated cation channel activity	5	101	1.3	0.0018
GO:0005261	Cation channel activity	7	326	0.94	0.0037
GO:0005515	Protein binding	32	7026	0.27	0.0037
GO:0005242	Inward rectifier potassium channel activity	3	20	1.79	0.0046
GO:0004905	Type i interferon receptor activity	2	2	2.61	0.0060
GO:0015077	Monovalent inorganic cation transmembrane transporter activity	7	381	0.87	0.0060
GO:0019962	Type i interferon binding	2	2	2.61	0.0060
GO:0035877	Death effector domain binding	2	3	2.43	0.0082
GO:0005251	Delayed rectifier potassium channel activity	3	30	1.61	0.0092
GO:0005102	Signaling receptor binding	13	1581	0.53	0.0104
GO:0022890	Inorganic cation transmembrane transporter activity	8	584	0.75	0.0104
GO:0044325	Ion channel binding	4	134	1.09	0.0375
GO:0015459	Potassium channel regulator activity	3	56	1.34	0.0411
GO:0005247	Voltage-gated chloride channel activity	2	11	1.87	0.0441
GO:0043422	Protein kinase b binding	2	11	1.87	0.0441
GO:0042802	Identical protein binding	13	1896	0.45	0.0463

Summary of findings on astroglia (discussion)

1) We observed 70 VS 158 DEGs that were altered between TBI APOE3/3 vs non demented control (NDC) APOE3/3 and TBI APOE4 carrier vs NDC APOE3/3 subjects, respectively (**See Figure 3 and Tables 1-2**). Therefore, signifying a worse outcome in astroglia transcriptomic profiles in APOE4+ TBI carriers vs APOE3/E3 carriers.
2) Most genes were downregulated in these two comparisons. No genes were upregulated in the TBI APOE3/E3 vs NDC APOE3/3 groups; however, we observed a few upregulated genes in the TBI APOE4+ vs NDC APOE3/E3 groups (**See Tables 1 and 2**).
3) There were more significantly differentiated genes that were unique to the (64) APOE4+ TBI vs NDC APOE3E3 comparison when compared to the (14) APOE3E3 TBI vs NDC APOE3E3 groups. Also, a significant number (42) of DEGs were found to overlap between the two comparisons. This suggests that although there are some similar underlying pathogenic processes driving by both genotypes, a noticeable distinct pathogenic process is observed in the APOE4 TBI compared to APOE3/E3 cases. This can also be seen in the number of DEG's (63) significantly altered between APOE4+ TBI vs APOE3E3 TBI cases (**See Figure 3 and Table 3**).
4) Some dysregulated pathways identified by reactome pathway analysis were similar between both comparisons above (e.g., altered micro-autophagy, signal transduction, G alpha signaling, calcium and sodium permeable ACH nicotinic receptor). There were also notable differences such as PKB signaling, TBC/RABGAPs, and Rab regulation of trafficking in the TBI APOE3/3 vs non demented control (NDC) APOE3/3 comparison, and

GABAB receptors, Apoptosis, Estrogen/ESR signaling events, TLR receptor cascades, PTK6 signaling, ERBB4 signaling and mitotic cell cycle in the TBI APOE4 carrier vs NDC APOE3/3 comparison (**Table 4 and 5**).

5) The changes observed when we compared TBI APOE4 carrier vs TBI APOE3/3 cases revealed alterations in different ion channel receptor and transporter activity and binding, Type I INF binding, PKB binding and death effector domain binding (**Table 6**).

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) Complete 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, for all the remainder of the planned study in Aim 1-3.

2) Complete administering injuries or sham injuries at 3 months of age for all the remainder of the planned studies in Aim 1-3. Animals will receive 2 closed head injuries each week for 3 months, using a CCI device.

4) Continue bioinformatic analyses of astrocytes and microglia gene array data in human tissue.

5) Generate astroglia RNAseq data in the mouse genotypes from the earliest timepoint of the study.

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 6 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 6 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. 12 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 has been completed, and bioinformatic analyses is currently ongoing and planned completion in the next quarter.

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)

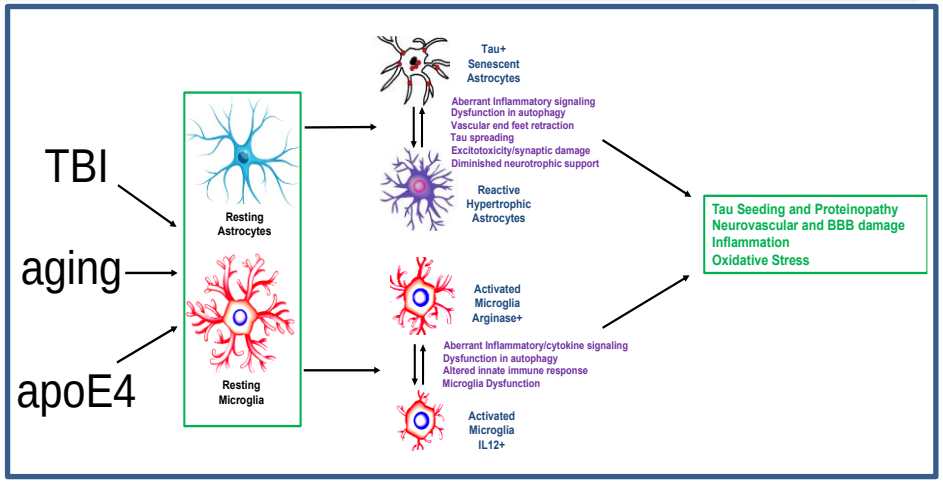
AIM 1: Delineating the influence of APOE genotype on pathological and biochemical outcomes 3 and 6 Mos after r-mTBI/sham in a GFAP-hTau mouse model with astrocyte specific (non-mutant) hTau expression.
AIM 2: To delineate the contribution of astrocytes to the spreading of Tau proteinopathies, and subsequent 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 and administering of injuries to APOE-TR and APOE-TR/GFAP-tau mice for planned studies in Aims 1 to 3.
Generated LCM/gene array autopsy data from microglia and astrocytes.

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
- COVID measures slowed down breeding activities
- Due to pathogen infection in transgenic mice obtained from our collaborator, mice rederivation services were used to generate a new colony of pathogen free mice.

Projected Expenditure: $441,658.00 **Actual Expenditure**: $358,351.49