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**TITLE:** Glial Cell Dysfunction in Neurodegenerative Sequelae of Repetitive Mild Traumatic Brain Injury

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**14. ABSTRACT**: The precise nature of how TBI leads to or precipitates ADRD pathogenesis is not understood. To address this problem we have, generated molecular profiles of AD and TBI pathogenesis in preclinical models at a range of ages/timepoints post-injury respectively, in order to identify molecules and pathways that are common to both AD and TBI and to correlate these with longitudinal changes in cognition and in the neuropathological landscape. Our analyses have highlighted the critical role of neuroinflammation after TBI, the particular significance of microglial responses. Understanding TBI neurodegeneration, and the triggers that encourage the pathogenic sequelae of TBI to follow paths toward ADRD, will be critical to the identification of effective therapeutic approaches. We have established and characterized a preclinical model of mTBI which has been validated by our clinical collaborators and which demonstrates lifelong neuroinflammatory responses and cognitive dysfunction following repetitive mTBI at a young age. We consider that repetitive mTBI induces significant and persistent changes in the microglial population over time after injury with lifelong consequences on the neuroinflammatory milieu. These microglial responses are critical to TBI neurodegeneration and in the context of other potentially pathogenic proteins such as tau or amyloid, ADRD pathobiology can be triggered.

**15. SUBJECT TERMS:** Glial cells, pathobiology, TBI, AD, Animal Models

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## 1. INTRODUCTION

A considerable lack of understanding still exists concerning the molecular mechanisms responsible for the pathogenic interrelationship between TBI and ADRD. Our preliminary data strongly implicate microglia pathobiology as a common denominator, and early event, and could represent an important molecular trigger for TBI mediated neurodegeneration and its increased risk for ADRD pathogenesis. Microglia are vital players of several physiological functions in the brain, and dysfunction of their supportive role can lead to the surrender of a host of these functions. This proposal will focus on the details of microglia pathobiology in mouse models and human cases of r-mTBI and ADRD proteinopathies. We will utilize a novel single cell array approach to interrogate the gene expression and transcript profiles in a homogenous population of resting and/or activated microglia. We will address how microglia pathobiology contributes to the relationship between TBI neurodegeneration and ADRD proteinopathies, and what role young vs aged microglia play in precipitating sequelae subsequent to TBI.

## 2. KEY WORDS: Glial cells, pathobiology, TBI, AD, Animal Models

## 3. KEY RESEARCH ACCOMPLISHMENTS

### OVERALL PROJECT SUMMARY

Traumatic Brain Injury (TBI), in particular mild TBI (mTBI) is a major cause of disability in military and in civilian populations, and for many years has been known to be an epigenetic risk factor for Alzheimer's Disease (AD) and other neurodegenerative conditions. However, the precise nature of how TBI leads to or precipitates AD or related dementia pathogenesis is not understood. To address this problem we have, in a previous PRARP contract, generated molecular profiles of AD and TBI pathogenesis in preclinical models at a range of ages/timepoints post-injury respectively, in order to identify molecules and pathways that are common to both AD and TBI and to correlate these with longitudinal changes in cognition and in the neuropathological landscape. Our analyses have highlighted the critical role of neuroinflammation after TBI, the particular significance of microglial responses, and the presence of unique and overlapping molecular pathways between TBI and AD models. These data suggesting a TBI neurodegeneration that is distinct from, but shares features with, known neurodegenerative conditions such as AD and AD related dementias (ADRD). Understanding TBI neurodegeneration itself, and the triggers that encourage the pathogenic sequelae of TBI to follow paths toward AD or ADRD, will be critical to the identification of effective therapeutic approaches. We have established and characterized a preclinical model of repetitive mTBI which has been validated by our clinical collaborators and which demonstrates lifelong neuroinflammatory responses and cognitive dysfunction following repetitive mTBI at a young age. Anti-inflammatory treatment paradigms in this preclinical model have demonstrated proof of concept for the significance of neuroinflammation by ameliorating both neuropathological and behavioral changes, but more sophisticated targeting is clearly needed to tackle the specific microglial responses which drive TBI pathobiology.

**Objective/Hypothesis:** Repetitive mTBI induces significant and persistent changes in the microglial population over time after injury with lifelong consequences on the neuroinflammatory milieu. These microglial responses are critical to TBI neurodegeneration and in the context of other potentially pathogenic proteins such as tau or amyloid, ADRD pathobiology can be triggered.

**Specific Aims:**
**Aim 1:** Delineation of the effects of chronic repetitive mTBI on microglia pathobiology and related proteinopathy in tau and amyloid bearing preclinical models at multiple time points post-injury, and validation in autopsied brains from human AD/CTE cases.
**Aim 2:** Generation of single cell genomic profiles in different populations of microglia obtained from preclinical models and validation in autopsied brains from human AD/CTE cases.

**The major goals of the project?**

**Major Task 1: Part One:** Delineation of the effects of chronic repetitive mTBI on microglia pathobiology and related proteinopathy in young and aged wild type, humanized tau and humanized amyloid mouse models at 24hrs, 3 months and 9 months post-injury **(Subtasks 1-2; 4-12). Part two:** Investigation of microglial pathological lesions in autopsied brains from human AD/CTE cases **(Subtask 3 and 12).**

Description: Histopathological assessment of microglia pathobiology in WT, hTau and $APP^{NLF}$mouse models exposed to r-mTBI, or sham injury (r-sham) when young (3 months – Young cohort) or aged (12 months – Aged cohort) and analyzed at 24hrs, 3 and 9 months post-injury. Histopathological assessment of microglia pathobiology and in human AD/TBI cases.

**Major Task 2: Part One:** Generation of single cell genomic profiles in different populations of microglia obtained from the same mouse models described in Aim 1 **(Subtask 2-5).**
**Part two:** Single cell genomic profiling of different population of microglia in autopsied brains from human AD/CTE cases **(Subtask 1 and 5).**

Description: Single cell genomic profiles in different populations of microglia obtained from young and aged WT, hTau, and $APP^{NLF}$ mice exposed to r-mTBI or r-sham and analyzed at 24hrs, 3 and 9 months post-injury. Validation of distinct microglial cell population of interest and identified gene profiles using flow cytometry and cell sorting. Single cell genomic profiles of microglia from autopsied human AD/CTE brains.

**SUMMARY OF ACCOMPLISHMENTS**

Our IACUC protocol was approved on the 2nd of August 2018. We have obtained regulatory approval for our ACURO protocol for this project on the 15th of November 2018. We have also obtained regulatory approval from our ORP Cadaver submission for use of human autopsy tissue on the 30th of October 2018.

We have applied to the NIH biobank to receive human TBI and/or AD autopsy brain tissue for neuropathological analyses. We have been assigned 58 fixed brain tissue samples. We have now received 42 samples to date, with 16 more being processed in the future (from the

Harvard brain bank). We are also seeking tissue from Boston Brain Bank for 20 more cases of repetitive mTBI. We are also going to combine these samples with previous tissue samples accrued in our Roskamp Brain Bank repository to increase sample size.

We have stained all 42 brain tissue samples with 7 different microglia antibodies – IBA1, MHCII, CD68, CD32A, CD163, CD206, and CD45, including double staining of IBA1 with 4G8 or PHF1. We are quantifying microglia in the gray and white matter for morphological phenotypes, immunoreactivity levels of their activation markers, cell density estimates, including their relationship to amyloid, tau and the vasculature. We are conducting these analyses on the 42 human tissue. No statistical analyses has been conducted to date as we do not have all the human cases required for the study. This will comment when all samples become available.

Our subcontractor will also begin conducting parallel single cell analyses of microglia from tissue sections in our AD, healthy control, and TBI groups. We have shipped all tissue sections (10 per case) to Dr. Mufson. He is conducting these studies over the next 3 months.

Mouse study – Comprises Young (3M) and Aged (12M) C57BL/6J, hTau and APP-KI mice. Aged Cohort: We have completed breeding of cohorts for Aged hTau and APP-KI mice for our TBI studies in Major Task 1 (36 mice per genotype - mixed gender). We now have all the numbers required for the Aged cohort from all genotypes (including C57BL/6J mice). We have begun the injuries on a fraction of these mice, with the balance of injuries to be completed by the first quarter of next year.

Young Cohort: We also began breeding cohorts for Young hTau and APP-KI mice for our TBI studies (36 mice per genotype – mixed gender). Injuries will be administered to all hTau and APP-KI mice within the next 3 months. All C57BL/6J wildtype mice requested for the project have been purchased, and exposed to their injuries. Mice from the 14day and 3 month timepoints have been euthanized.

Immunohistochemistry, immunofluorescence, electron microscopy and single cell array will commence after mice per genotype from all timepoints have been euthanized. We anticipate generation of data from the young cohort beginning in March 2020.

**What have we accomplished under the Major goals?**

As detailed above, we have accomplished a major part of subtasks 1 to subtask 5, related to MAJOR TASK 1 (please see below for the list of subtasks relating to both MAJOR TASKS 1 and 2). Our collaborator is also in the process of conducting single cell array analyses of autopsy tissue for subtask 1, related to MAJOR TASK 2.

SUBTASK DESCRIPTIONS FOR MAJOR TASK 1

**Subtask 1**: Obtaining ACURO approval and submitting ORP Cadaver form.
**We have obtained regulatory approval for both animal and autopsy tissue studies.**
**Substask 2a**: Breeding of cohorts for Aged mice TBI studies (36 mice per genotype - mixed gender).
**We have completed breeding cohorts of Aged mice.**
**Subtask 2b**: Breeding of cohorts for Young mice TBI studies (36 mice per genotype – mixed gender).
**We have almost completed breeding cohorts of Young mice.**

**Subtask 3**: Histopathological analyses of microglia pathobiology in human AD/CTE cases (n=80; 10/group with 8 different groups).

**Completed for 42 brain samples.**

**Subtask 4:** Administering injuries to Young cohort (36 per genotype).
Mice used will be mixed gender WT, hTau, and $APP^{NLF}$ transgenic mice on the C57BL/6 background, aged 3 months at injury. Animals will receive 5 closed head injuries/week with a CCI device over a 1-month period (Ojo et al., 2016). For each of the three genotypes there will be two groups - r-mTBI young and r-sham young - each with 18 mice per group. Of these 18 mice, 6 will be euthanized for analysis at each of the three timepoints.

**Completed for C57BL/6J mice, ongoing for hTau and APP-KI mice.**

**Subtask 5:** Euthanasia of Young cohort; for each of the three genotypes (WT, hTau, and $APP^{NLF}$) there will be 36 mice, of which 18 will have received r-mTBI and 18 received r-sham. For each genotype, 6 r-mTBI and 6 r-sham mice will be euthanized at 1 day post-injury, 3 months post-injury and 9 months post-injury.

**Completed for 2/3rd of Young C57BL6 mice.**

**Subtask 6:** Sectioning and histopathological staining of Young cohort tissues with microglia, tau and amyloid antibodies; and TUNEL, BrdU, EM preps.

**No started – Plan to begin in March 2020**

**Subtask 7:** Stereological analyses and Image quantitation of brain sections from Young cohort (staggered over time)

**No started – Plan to begin after March 2020**

**Subtask 8:** Administering injuries to Aged cohort (36 per genotype).
Mice used will be mixed gender WT, hTau, and $APP^{NLF}$ transgenic mice on the C57BL/6 background, aged 12 months at injury.

**Began and ongoing**

**Subtask 9:** Euthanasia of Aged cohort; for each of the three genotypes (WT, hTau, and $APP^{NLF}$) there will be 36 mice, of which 18 will have received r-mTBI and 18 received r-sham. For each genotype, 6 r-mTBI and 6 r-sham mice will be euthanized at 1 day post-injury, 3 months post-injury and 9 months post-injury.

**A fraction of hTau mice have been injured**

**Subtask 10:** Sectioning and histopathological staining of Aged cohort tissues with microglia, tau and amyloid antibodies; and TUNEL, BrdU, EM preps.

**Not started**

**Subtask 11:** Stereological analyses and Image quantitation of brain sections from Aged cohort

**Not started**

**Subtask 12:** Interpretation of data and consultation with clinical neuropathologists

**Begin once new set of data have been acquired**

SUBTASK DESCRIPTIONS FOR MAJOR TASK 2

**Subtask 1:** Laser capture microdissection of microglia from human AD/CTE cases for single cell array for gene expression profiling.

**Ongoing with Barrow (Dr Mufson)**

**Subtask 2:** Laser capture microdissection of microglia from Young Cohort WT, hTau, and $APP^{NLF}$ mice for single cell array for gene expression profiling (staggered overtime).

**Subtask 3:** Laser capture microdissection of microglia from Aged Cohort WT, hTau, and $APP^{NLF}$ mice for single cell array for gene expression profiling (staggered overtime).

**Subtask 4:** Validation of select gene transcript profiles that are altered in microglia in both Young and Aged cohorts.

**Subtask 5:** Data analysis and interpretation and correlation studies

## BELOW IS A SUMMARY OF THE MAIN FINDINGS FROM OUR STUDIES THIS YEAR

## METHODOLOGY

**Immunostaining:** Briefly, sections were deparaffinized in xylene and rehydrated in a decreasing gradient of ethanol before the IHC procedure. Sections were rinsed in distilled water and subsequently incubated at room temperature in a solution of endogenous peroxidase blocking solution containing 3% hydrogen peroxide diluted in PBS for 15 minutes. For primary antibodies requiring antigen retrieval, sections were treated with either (I) DAKO target retrieval citrate buffer solution (pH 6) (Dako) for 8 minutes in the microwave, or (II) Tris-ethylene-diamine-tetra acetic acid—Tris-EDTA buffer (pH 8). Following antigen retrieval, sections were blocked for 1 hour in Dako protein serum-free protein block (Dako). Sections were immunostained in batches with primary antibodies made up in supersensitive wash buffer and antibody diluent background-reducing agent. After overnight incubation, sections were rinsed with PBS and transferred to a solution containing the appropriate conjugated (peroxidase or alkaline phosphatase) secondary antibody (Vectastain Elite ABC Kit, Vector Laboratories) for 1 hour, depending on the specific requirement of the antibody protocol. Immunoreactivity was visualized with a compatible chromogen. Development with the chromogen was timed and applied as a constant across batches to limit technical variability before progressing to quantitative image analysis. Mounted sections were progress through a graded series of alcohols (dehydrated), cleared in xylene, and cover-slipped with permanent mounting medium. Immunoreacted sections were viewed using a motorized Olympus (BX63) upright microscope and photographs taken using the high-resolution DP72 color digital camera. **Image Analysis:** Rigorous staining protocols were applied to ensure consistency of staining and accuracy of image analysis. Immunoreactivity for cell markers were measured by quantitative image analysis performed blind by investigator. Multiple ROI's were analyzed in standardized fashion for each marker. A survey of immunostained tissue sections was performed independently to verify specific immunoreactivity that will be subsequently be used for quantitative image analysis. Briefly, non-overlapping red, green, blue (RGB) images were digitally captured randomly within the defined areas from each section, providing a systematic survey of each region of interest for each animal within a group. A minimum of 60 microscopic fields (×40 magnification) were analyzed per region. Immunoreacted profiles were optically segmented and analyzed using CellSens morphometric image analysis software (Olympus, Center Valley, Pennsylvania). A semi-automated RGB histogram-based protocol (specified in the image analysis program) was used to determine the optimal segmentation (threshold setting) for immunoreactivity for each antibody. Immunoreactive profiles discriminated in this manner were used to determine the specific immunoreactive percentage area estimates. **Statistical analyses:** Data has not been analyzed as we are accruing more samples to complete the groups. We have presented the data here for the purpose of reporting our data and progress on the project.

**Gene transcript profiling:** RNA extraction and NanoString quantification. Total RNA was extracted from frozen tissue samples using RNA Isolation Kit according to the manufacturer's protocol. Concentrations of extracted RNA were determined using the NanoDrop Spectrophotometer. Samples with RNA concentrations of <40 ng/μL, A260/A280 ratios <1.5 or A260/230 ratios <1.0 were considered as inadequate and were excluded from the analysis. A NanoString panel was designed, comprising 770 previously published inflammatory genes. Additionally, five housekeeping genes showing minimal alteration across samples were also

included as controls. The custom-designed probes included a 100-bp region targeting the mRNA, with two sequence-specific, fluorescent-barcoded probes for each target (3' biotinylated capture probe and a 5' reporter probe). Probes and 100 ng total RNA were hybridised overnight at 65°C according to the manufacturer's protocol. A NanoString nCounter Digital Analyzer (NanoString Technologies, Seattle, Washington, USA) was used to count the digital barcodes representing the number of transcripts. The raw expression data were normalised using nSolver Analysis software. A normalisation factor was calculated by obtaining the geometric mean of the positive controls used for each sample and applied to the raw counts of the nCounter output data to eliminate variability that was unrelated to the samples. The resulting data were normalised again with the geometric mean of the housekeeping genes. Normalised data were $log_2$-transformed for further analyses. **Statistical analysis** - The normalised $log_2$-transformed mRNA expression data were analysed by unsupervised hierarchical clustering using Cluster V.3.0 and Java Tree view software. Heat maps showing high and low expression of genes in the subtypes were generated and categorized on the basis of these expression patterns. Statistical analysis was performed between TBI vs control groups using T-Test, and a p value $<0.05$ was considered significant. All p values were adjusted using a false discovery rate (FDR) correction of 5%. All tests were two-sided at the significance level $p<0.05$.

**Microglia analyses in autopsy tissue**

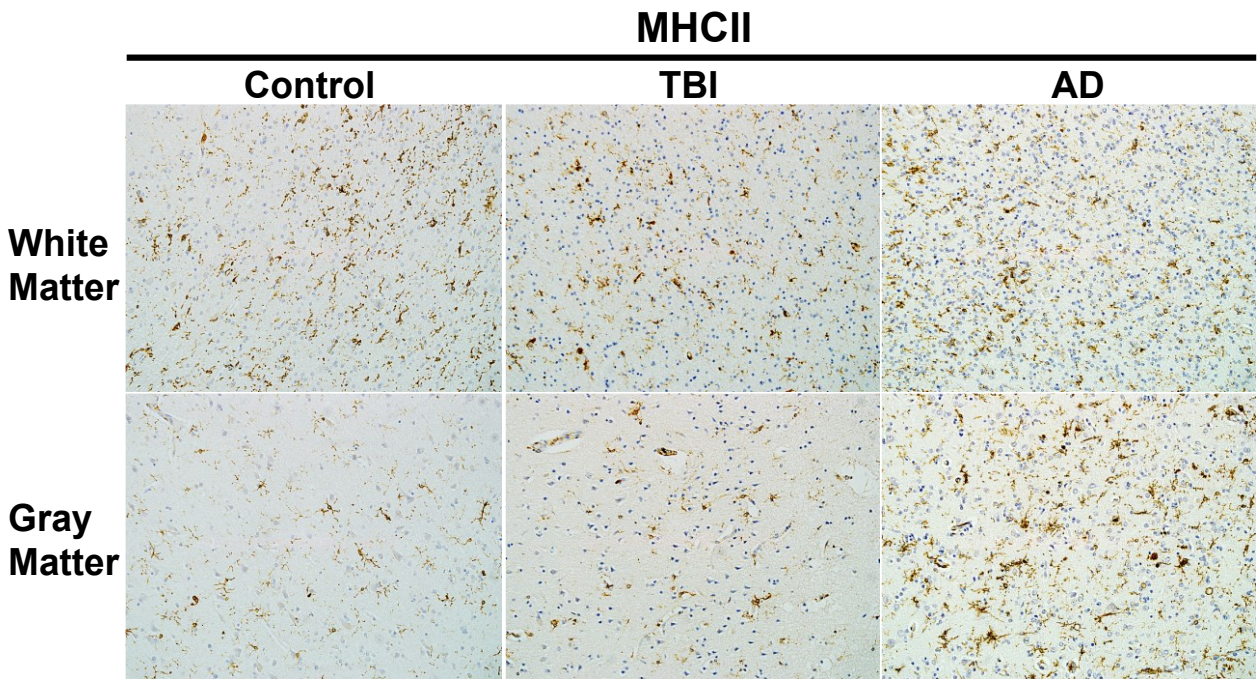
**Table 1 shows group classification and history of de-identified patients analyzed for microglia markers.**

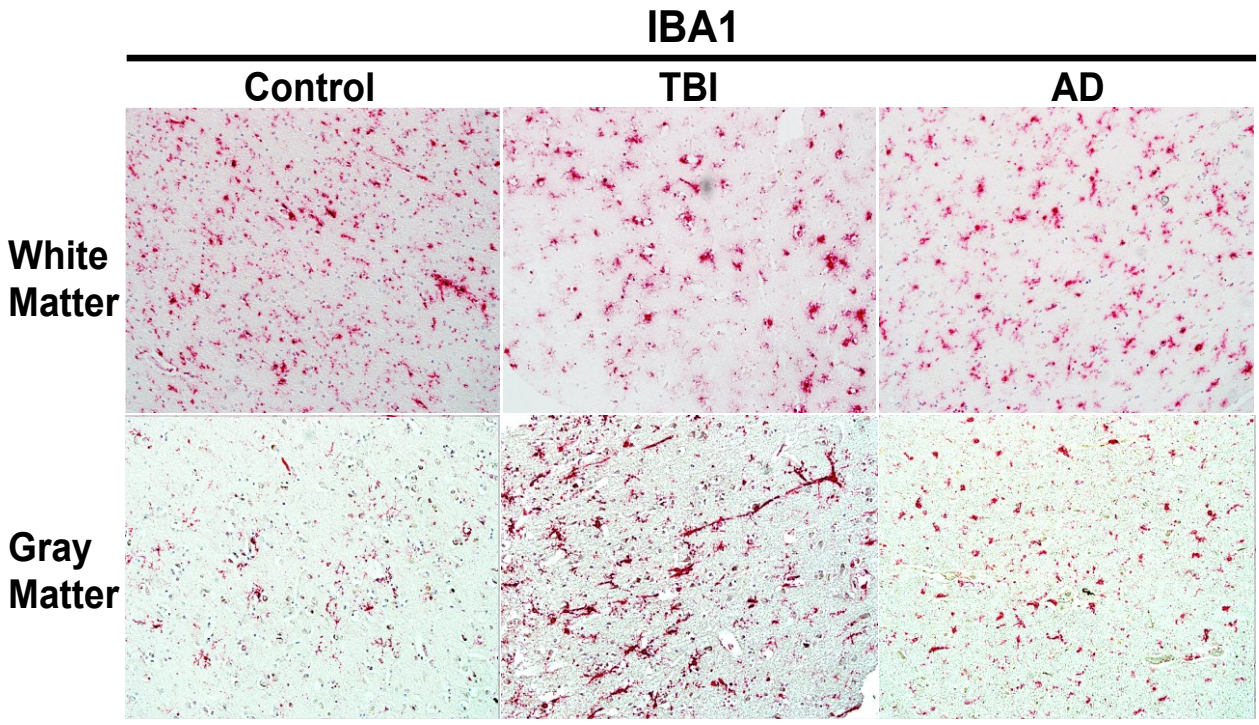
**Table 1:** Group Classification and History

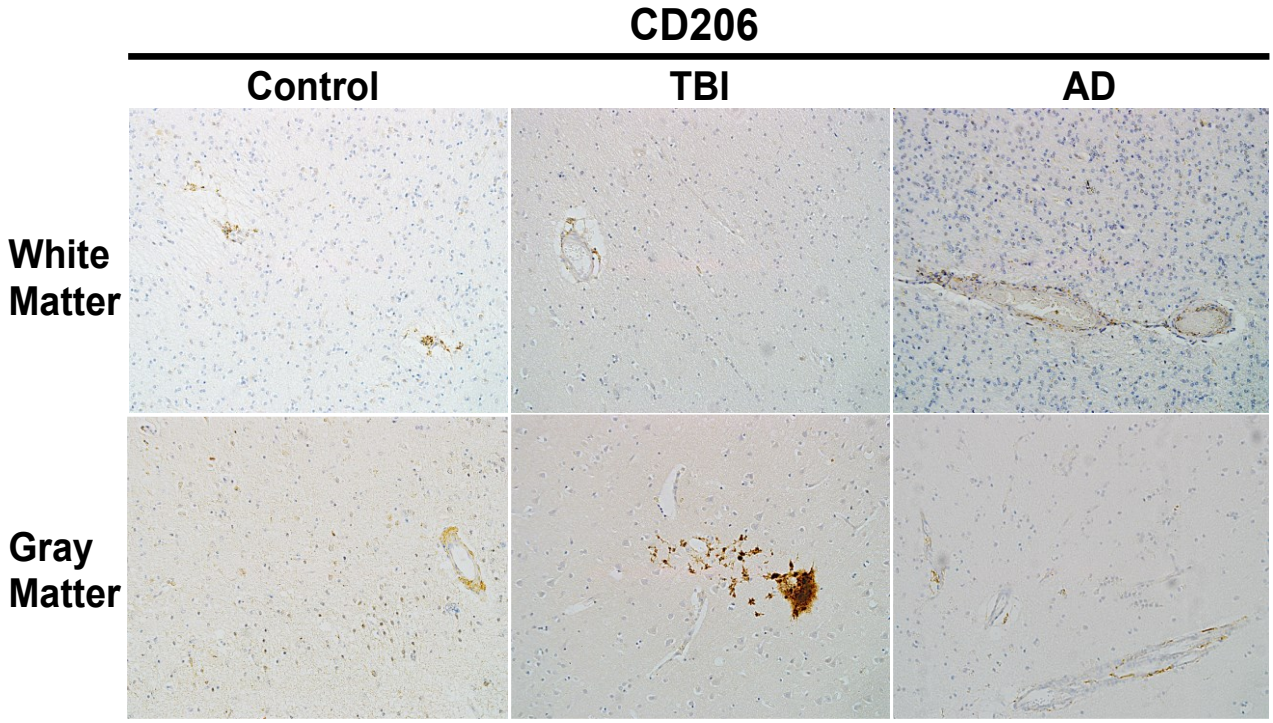
| Disease status | Age (yrs) | CDR | Plaque count | Braak Stage |
|---|---|---|---|---|
| **Low CDR/Plaque AD** | 93.00 | 0.30 | 5.21 | 4.80 |
| **High CDR/Braak AD** | 76.91 | 2.36 | 18.14 | 6.00 |
| **Non Demented** | 79.64 | 0.09 | 0.15 | 1.00 |
| **Repetitive MTBI** | 61.86 | - | N/A | N/A |

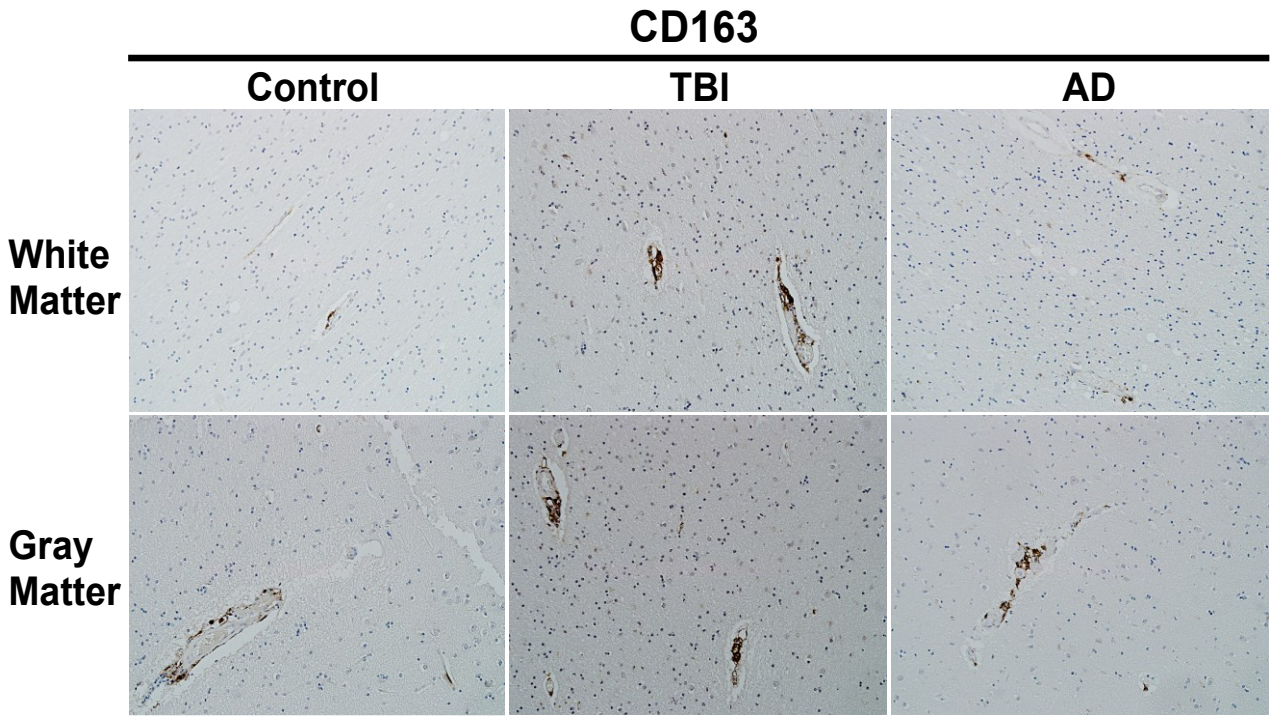
**Note: TBI patients presented herein have evidence of 2 or more repetitive mild traumatic brain injuries with several decades post-survival.**

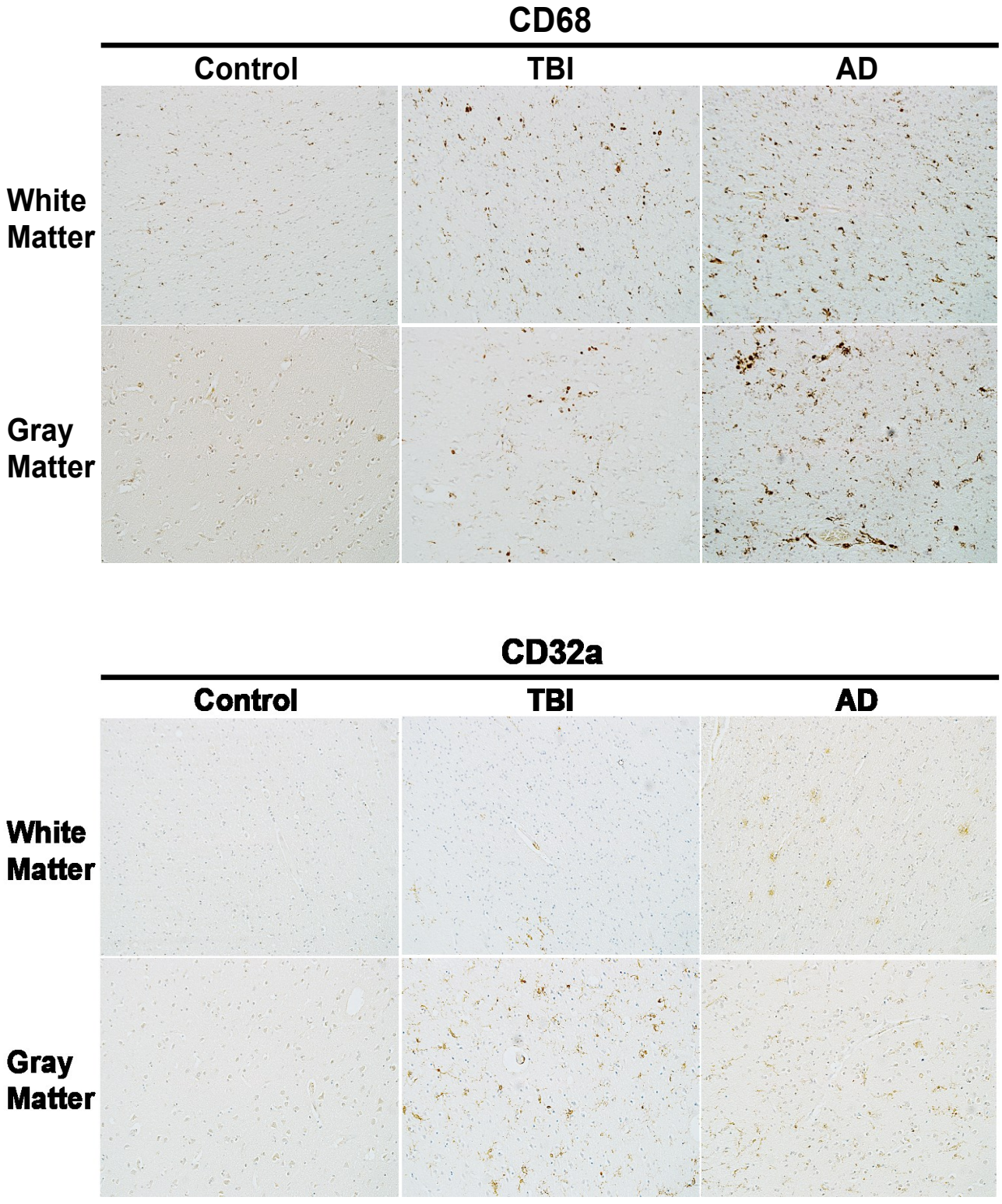
**Micrographs below depict qualitative changes using different microglia markers.**




CD206
Control
TBI
AD
White Matter
Gray Matter


CD163
Control
TBI
AD
White Matter
Gray Matter


CD68
Control
TBI
AD
White Matter
Gray Matter
CD32a
Control
TBI
AD
White Matter
Gray Matter

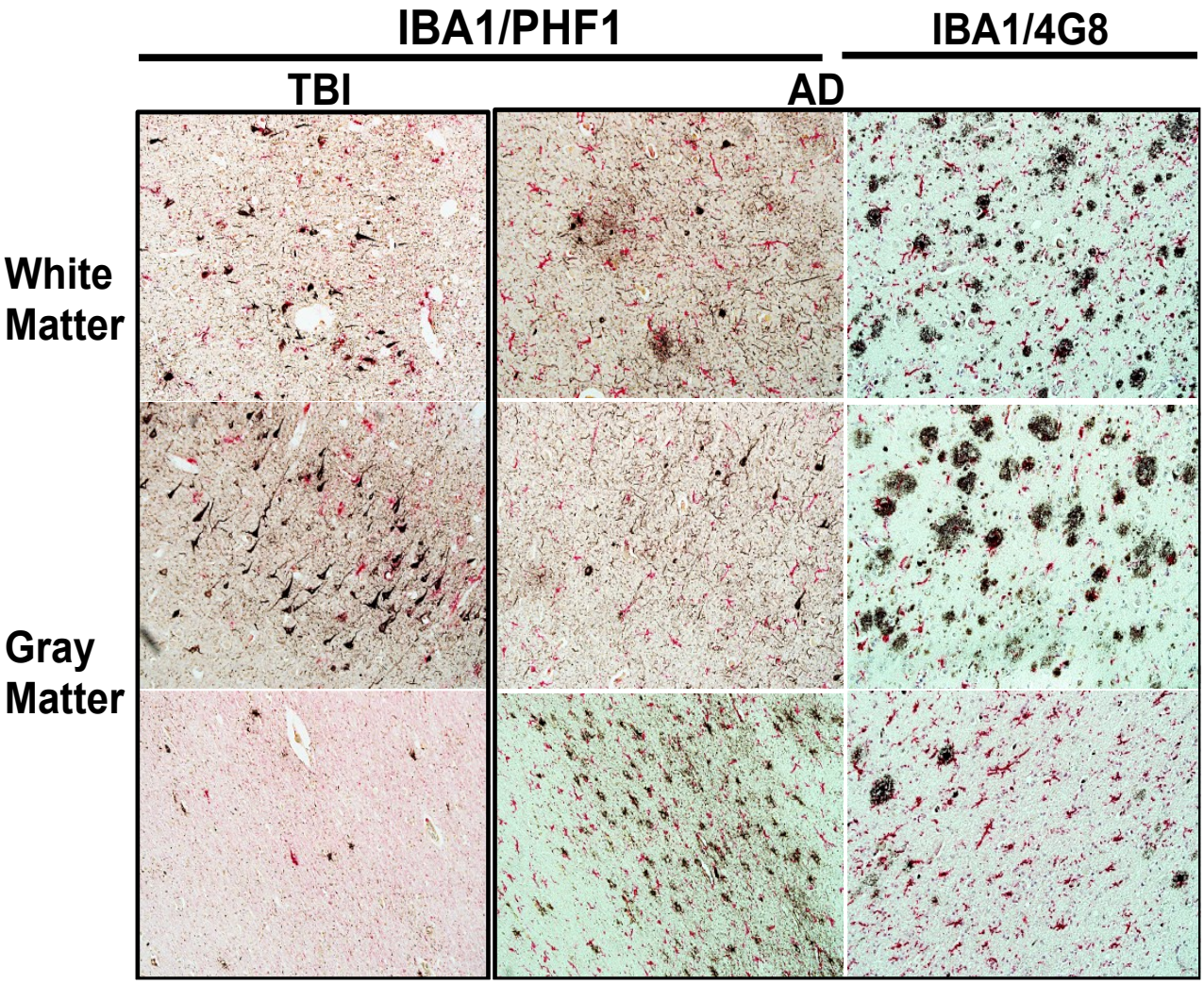

IBA1/PHF1
IBA1/4G8
TBI
AD
White Matter
Gray Matter

**Figure Two shows qualitative changes in some of the different microglia markers analyzed between AD and control cases**

Qualitative analyses of brain tissue from healthy control and AD patients is shown in Figure 2 below. We have not included quantitative analyses of TBI brains as we are still waiting for more samples from the brain bank. Our qualitative analyses demonstrates some notable differences and trends in CD68, IBA1, MHCII immune markers, and perivascular immune markers CD163 and CD206 between control and AD brains. These preliminary data are shown for the purpose of reporting our progress in this quarter for our project. More data will be incorporated at a later date once we have a complete sample size per group.

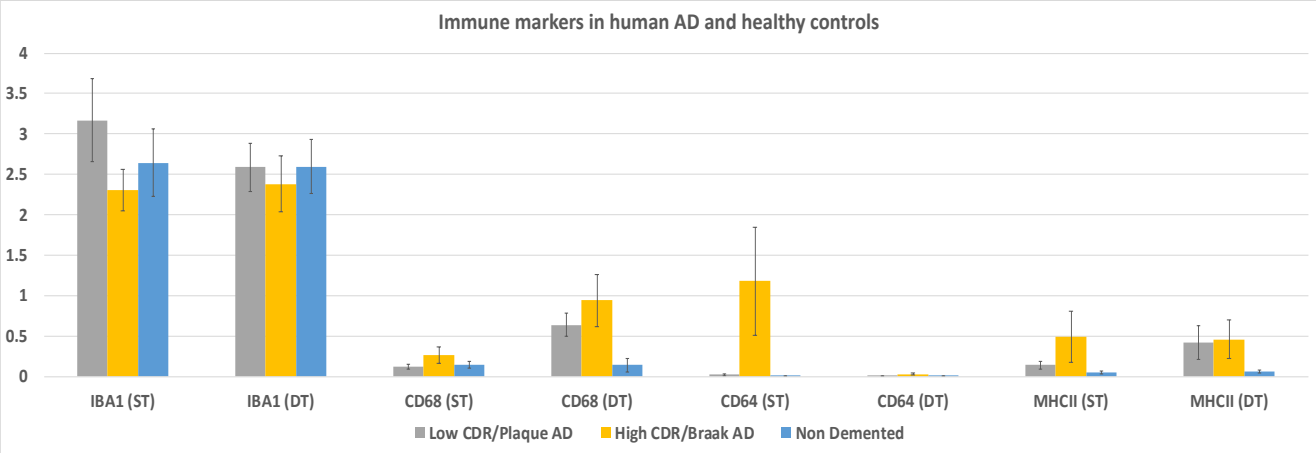



**Figure 2:** Graph showing microglia markers in the grey and white matter tissue of AD and healthy control brain samples from the BM21

**NanoString data generated from a study consisting of healthy controls, AD and TBI autopsy cases from the BA9 region. We have presented and summarized aspects of microglia pathobiology related genes changing between control and injured brain tissue.**

## Control vs AD - Autophagy

| Probe Label | Log2 fold change | std error (log2) | P-value | Gene.sets |
|---|---|---|---|---|
| RB1CC1-mRNA | -0.737 | 0.174 | 0.00216 | Autophagy, Cellular Stress |
| PIK3CA-mRNA | -0.577 | 0.168 | 0.00737 | Adaptive Immune Response, Angiogenesis, Apoptosis, Autophagy, Carbohydrate Metabolism, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, Insulin Signaling, Lipid Metabolism |
| MFGE8-mRNA | 0.712 | 0.222 | 0.0108 | Autophagy |
| CD47-mRNA | -0.748 | 0.242 | 0.0129 | Autophagy, Matrix Remodeling |
| PTEN-mRNA | -0.743 | 0.243 | 0.0135 | Adaptive Immune Response, Autophagy, DNA Damage, Growth Factor Signaling, Lipid Metabolism |
| ATG14-mRNA | -0.814 | 0.271 | 0.0147 | Autophagy, Cellular Stress |
| CD36-mRNA | -1.42 | 0.509 | 0.0208 | Adaptive Immune Response, Autophagy, Innate Immune Response, Matrix Remodeling, Neurons and Neurotransmission |
| STX18-mRNA | -0.637 | 0.23 | 0.0219 | Autophagy |
| RALB-mRNA | -0.648 | 0.24 | 0.0246 | Autophagy, Growth Factor Signaling |
| ATG5-mRNA | -0.75 | 0.282 | 0.026 | Autophagy, Cellular Stress, Innate Immune Response |
| PRKACB-mRNA | -0.742 | 0.287 | 0.0295 | Adaptive Immune Response, Angiogenesis, Apoptosis, Autophagy, Growth Factor Signaling, Lipid Metabolism, Neurons and Neurotransmission, Wnt |
| PLA2G4A-mRNA | -1.07 | 0.417 | 0.0306 | Autophagy, Cellular Stress, Growth Factor Signaling, Lipid Metabolism, Oligodendrocyte Function |
| HMGB1-mRNA | -0.887 | 0.368 | 0.0393 | Apoptosis, Autophagy, Inflammatory Signaling, Innate Immune Response |
| ATG3-mRNA | -0.596 | 0.25 | 0.0409 | Autophagy, Cellular Stress |
| VAMP7-mRNA | -0.644 | 0.278 | 0.0455 | Autophagy, Neurons and Neurotransmission |

## Control vs TBI - Autophagy

| Probe Label | Log2 fold change | std error (log2) | P-value | Gene.sets |
|---|---|---|---|---|
| PIK3CA-mRNA | -0.965 | 0.192 | 0.000704 | Adaptive Immune Response, Angiogenesis, Apoptosis, Autophagy, Carbohydrate Metabolism, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, Insulin Signaling, Lipid Metabolism |
| RB1CC1-mRNA | -0.969 | 0.198 | 0.000854 | Autophagy, Cellular Stress |
| PIK3R1-mRNA | -1.45 | 0.297 | 0.000868 | Adaptive Immune Response, Angiogenesis, Apoptosis, Autophagy, Carbohydrate Metabolism, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, Insulin Signaling, Lipid Metabolism |
| PIK3CB-mRNA | -0.875 | 0.189 | 0.00122 | Adaptive Immune Response, Angiogenesis, Apoptosis, Autophagy, Carbohydrate Metabolism, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, Insulin Signaling, Lipid Metabolism |
| CLN3-mRNA | 1.14 | 0.316 | 0.00559 | Autophagy |
| PRKACB-mRNA | -1.08 | 0.327 | 0.00929 | Adaptive Immune Response, Angiogenesis, Apoptosis, Autophagy, Growth Factor Signaling, Lipid Metabolism, Neurons and Neurotransmission, Wnt |

| | | | | |
|---|---|---|---|---|
| SFTPD-mRNA | -1.95 | 0.641 | 0.0142 | Adaptive Immune Response, Autophagy, Innate Immune Response |
| VPS4B-mRNA | -0.866 | 0.286 | 0.0143 | Autophagy, Neurons and Neurotransmission |
| STX18-mRNA | -0.787 | 0.262 | 0.015 | Autophagy |
| ATG14-mRNA | -0.923 | 0.308 | 0.0151 | Autophagy, Cellular Stress |
| MAP1LC3A-mRNA | -1.19 | 0.414 | 0.018 | Autophagy, Cellular Stress |
| MAPK10-mRNA | -1.35 | 0.468 | 0.0181 | Adaptive Immune Response, Apoptosis, Autophagy, Cellular Stress, Growth Factor Signaling, Innate Immune Response, Wnt |
| CD14-mRNA | 1.17 | 0.423 | 0.0219 | Apoptosis, Astrocyte Function, Autophagy, Growth Factor Signaling, Innate Immune Response, NF-kB |
| WAS-mRNA | 1 | 0.364 | 0.0224 | Adaptive Immune Response, Autophagy |
| PRKACA-mRNA | -1.05 | 0.382 | 0.0224 | Adaptive Immune Response, Angiogenesis, Apoptosis, Autophagy, Cell Cycle, Cytokine Signaling, Growth Factor Signaling, Lipid Metabolism, Neurons and Neurotransmission, Wnt |
| RALB-mRNA | -0.752 | 0.274 | 0.0226 | Autophagy, Growth Factor Signaling |
| HMGB1-mRNA | -1.14 | 0.42 | 0.0234 | Apoptosis, Autophagy, Inflammatory Signaling, Innate Immune Response |
| PTEN-mRNA | -0.751 | 0.276 | 0.0236 | Adaptive Immune Response, Autophagy, DNA Damage, Growth Factor Signaling, Lipid Metabolism |
| CD47-mRNA | -0.735 | 0.276 | 0.0257 | Autophagy, Matrix Remodeling |
| RALA-mRNA | -0.89 | 0.337 | 0.027 | Autophagy, Growth Factor Signaling, Neurons and Neurotransmission |
| MFGE8-mRNA | 0.646 | 0.253 | 0.0311 | Autophagy |
| SQSTM1-mRNA | 0.631 | 0.25 | 0.0326 | Autophagy, Cytokine Signaling, Growth Factor Signaling |
| ULK1-mRNA | 0.892 | 0.366 | 0.0373 | Autophagy, Cellular Stress |
| AXL-mRNA | 0.878 | 0.366 | 0.0401 | Angiogenesis, Autophagy, Microglia Function |
| VAMP7-mRNA | -0.728 | 0.316 | 0.0468 | Autophagy, Neurons and Neurotransmission |
| IQSEC1-mRNA | -0.538 | 0.236 | 0.0484 | Autophagy |

## Control vs AD – Cytokine signaling

| Probe Label | Log2 fold change | std error (log2) | P-value | Gene.sets |
|---|---|---|---|---|
| IL1R2-mRNA | 3.01 | 0.66 | 0.00137 | Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling |
| CCL4-mRNA | -2.7 | 0.781 | 0.00718 | Cytokine Signaling, Innate Immune Response, Microglia Function, NF-kB |
| PIK3CA-mRNA | -0.577 | 0.168 | 0.00737 | Adaptive Immune Response, Angiogenesis, Apoptosis, Autophagy, Carbohydrate Metabolism, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, Insulin Signaling, Lipid Metabolism |
| IL1B-mRNA | -2.23 | 0.702 | 0.0112 | Apoptosis, Cellular Stress, Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response, Microglia Function, NF-kB |
| SUMO1-mRNA | -1.13 | 0.391 | 0.0181 | Cell Cycle, Cytokine Signaling, DNA Damage, Inflammatory Signaling |
| CCL3-mRNA | -2.47 | 0.959 | 0.0297 | Cytokine Signaling, Microglia Function |
| BRAF-mRNA | -0.408 | 0.159 | 0.03 | Adaptive Immune Response, Angiogenesis, Apoptosis, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, Insulin Signaling, Neurons and Neurotransmission |
| LTB-mRNA | -1.4 | 0.543 | 0.0301 | Cytokine Signaling, Inflammatory Signaling, NF-kB |
| TNFSF10-mRNA | -1.2 | 0.48 | 0.0337 | Apoptosis, Cytokine Signaling, Innate Immune Response |
| IFNAR1-mRNA | -0.588 | 0.236 | 0.0343 | Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response |
| CDKN1A-mRNA | 2.41 | 0.983 | 0.0366 | Adaptive Immune Response, Cell Cycle, Cellular Stress, Cytokine Signaling, DNA Damage, Growth Factor Signaling |

## Control vs TBI – Cytokine Signaling

| Probe Label | Log2 fold change | std error (log2) | P-value | Gene.sets |
|---|---|---|---|---|
| PIK3CA-mRNA | -0.965 | 0.192 | 0.000704 | Adaptive Immune Response, Angiogenesis, Apoptosis, Autophagy, Carbohydrate Metabolism, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, Insulin Signaling, Lipid Metabolism |
| PIK3R1-mRNA | -1.45 | 0.297 | 0.000868 | Adaptive Immune Response, Angiogenesis, Apoptosis, Autophagy, Carbohydrate Metabolism, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, Insulin Signaling, Lipid Metabolism |
| PIK3CB-mRNA | -0.875 | 0.189 | 0.00122 | Adaptive Immune Response, Angiogenesis, Apoptosis, Autophagy, Carbohydrate Metabolism, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, Insulin Signaling, Lipid Metabolism |
| BRAF-mRNA | -0.654 | 0.181 | 0.0056 | Adaptive Immune Response, Angiogenesis, Apoptosis, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, Insulin Signaling, Neurons and Neurotransmission |
| LTB-mRNA | -2 | 0.634 | 0.0116 | Cytokine Signaling, Inflammatory Signaling, NF-kB |
| NGFR-mRNA | 1.84 | 0.587 | 0.012 | Apoptosis, Cellular Stress, Cytokine Signaling, Growth Factor Signaling |
| SUMO1-mRNA | -1.38 | 0.445 | 0.0128 | Cell Cycle, Cytokine Signaling, DNA Damage, Inflammatory Signaling |
| TNFRSF13C-mRNA | -1.62 | 0.582 | 0.021 | Cytokine Signaling, Inflammatory Signaling, NF-kB |
| PRKACA-mRNA | -1.05 | 0.382 | 0.0224 | Adaptive Immune Response, Angiogenesis, Apoptosis, Autophagy, Cell Cycle, Cytokine Signaling, Growth Factor Signaling, Lipid Metabolism, Neurons and Neurotransmission, Wnt |
| NEFL-mRNA | -1.78 | 0.661 | 0.0244 | Adaptive Immune Response, Angiogenesis, Cytokine Signaling, Growth Factor Signaling, Insulin Signaling, Neurons and Neurotransmission |
| KIT-mRNA | -1.15 | 0.428 | 0.0244 | Adaptive Immune Response, Angiogenesis, Cytokine Signaling, Growth Factor Signaling, Insulin Signaling |
| IRAK1-mRNA | 1.27 | 0.476 | 0.026 | Apoptosis, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, NF-kB |
| GRIN2A-mRNA | -0.694 | 0.269 | 0.0296 | Adaptive Immune Response, Angiogenesis, Cytokine Signaling, Growth Factor Signaling, Insulin Signaling, Neurons and Neurotransmission |
| RELB-mRNA | 1.9 | 0.75 | 0.0317 | Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling, NF-kB |
| SQSTM1-mRNA | 0.631 | 0.25 | 0.0326 | Autophagy, Cytokine Signaling, Growth Factor Signaling |
| IFNAR2-mRNA | -0.932 | 0.396 | 0.043 | Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response |
| ERBB3-mRNA | 1.25 | 0.533 | 0.0433 | Adaptive Immune Response, Angiogenesis, Cytokine Signaling, Growth Factor Signaling, Insulin Signaling, Oligodendrocyte Function |
| IFNAR1-mRNA | -0.619 | 0.269 | 0.0467 | Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response |
| TRAF2-mRNA | 1.02 | 0.443 | 0.0472 | Apoptosis, Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response, NF-kB |

## Control vs AD – Inflammatory Signaling

| Probe Label | Log2 fold change | std error (log2) | P-value | Gene.sets |
|---|---|---|---|---|
| IL1R2-mRNA | 3.01 | 0.66 | 0.00137 | Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling |
| IL1B-mRNA | -2.23 | 0.702 | 0.0112 | Apoptosis, Cellular Stress, Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response, Microglia Function, NF-kB |
| MPEG1-mRNA | -1.44 | 0.471 | 0.0138 | Inflammatory Signaling |
| NFKBIA-mRNA | 1.1 | 0.365 | 0.0146 | Adaptive Immune Response, Apoptosis, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response, NF-kB |
| C5AR1-mRNA | 2.84 | 0.957 | 0.0157 | Inflammatory Signaling, Neurons and Neurotransmission |
| SUMO1-mRNA | -1.13 | 0.391 | 0.0181 | Cell Cycle, Cytokine Signaling, DNA Damage, Inflammatory Signaling |
| LTB-mRNA | -1.4 | 0.543 | 0.0301 | Cytokine Signaling, Inflammatory Signaling, NF-kB |
| CYP7B1-mRNA | -0.696 | 0.271 | 0.0303 | Inflammatory Signaling |
| GBP2-mRNA | 2.58 | 1 | 0.0305 | Astrocyte Function, Inflammatory Signaling |
| LILRB4-mRNA | -1.14 | 0.456 | 0.0339 | Adaptive Immune Response, Inflammatory Signaling |

| | | | | |
|---|---|---|---|---|
| IFNAR1-mRNA | -0.588 | 0.236 | 0.0343 | Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response |
| HMGB1-mRNA | -0.887 | 0.368 | 0.0393 | Apoptosis, Autophagy, Inflammatory Signaling, Innate Immune Response |

## Control vs TBI – Inflammatory Signaling

| Probe Label | Log2 fold change | std error (log2) | P-value | Gene.sets |
|---|---|---|---|---|
| LTB-mRNA | -2 | 0.634 | 0.0116 | Cytokine Signaling, Inflammatory Signaling, NF-kB |
| SUMO1-mRNA | -1.38 | 0.445 | 0.0128 | Cell Cycle, Cytokine Signaling, DNA Damage, Inflammatory Signaling |
| TNFRSF13C-mRNA | -1.62 | 0.582 | 0.021 | Cytokine Signaling, Inflammatory Signaling, NF-kB |
| HMGB1-mRNA | -1.14 | 0.42 | 0.0234 | Apoptosis, Autophagy, Inflammatory Signaling, Innate Immune Response |
| CD74-mRNA | 1.41 | 0.52 | 0.024 | Adaptive Immune Response, Inflammatory Signaling |
| RELB-mRNA | 1.9 | 0.75 | 0.0317 | Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling, NF-kB |
| MPEG1-mRNA | -1.3 | 0.536 | 0.0385 | Inflammatory Signaling |
| IFNAR2-mRNA | -0.932 | 0.396 | 0.043 | Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response |
| IFNAR1-mRNA | -0.619 | 0.269 | 0.0467 | Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response |
| TRAF2-mRNA | 1.02 | 0.443 | 0.0472 | Apoptosis, Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response, NF-kB |
| BIN1-mRNA | 0.795 | 0.35 | 0.0493 | Inflammatory Signaling |
| IFI30-mRNA | 1.6 | 0.704 | 0.0495 | Adaptive Immune Response, Inflammatory Signaling |

## Control vs AD – Innate immune Response

| Probe Label | Log2 fold change | std error (log2) | P-value | Gene.sets |
|---|---|---|---|---|
| PPP3R1-mRNA | -0.826 | 0.211 | 0.00354 | Adaptive Immune Response, Apoptosis, Growth Factor Signaling, Innate Immune Response, Wnt |
| CCL4-mRNA | -2.7 | 0.781 | 0.00718 | Cytokine Signaling, Innate Immune Response, Microglia Function, NF-kB |
| PIK3CA-mRNA | -0.577 | 0.168 | 0.00737 | Adaptive Immune Response, Angiogenesis, Apoptosis, Autophagy, Carbohydrate Metabolism, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, Insulin Signaling, Lipid Metabolism |
| BID-mRNA | -0.578 | 0.176 | 0.00961 | Apoptosis, DNA Damage, Innate Immune Response |
| IL1B-mRNA | -2.23 | 0.702 | 0.0112 | Apoptosis, Cellular Stress, Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response, Microglia Function, NF-kB |
| NFKBIA-mRNA | 1.1 | 0.365 | 0.0146 | Adaptive Immune Response, Apoptosis, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response, NF-kB |
| CASP3-mRNA | -0.683 | 0.238 | 0.0185 | Apoptosis, Cellular Stress, DNA Damage, Growth Factor Signaling, Innate Immune Response, Matrix Remodeling |
| CD36-mRNA | -1.42 | 0.509 | 0.0208 | Adaptive Immune Response, Autophagy, Innate Immune Response, Matrix Remodeling, Neurons and Neurotransmission |
| XIAP-mRNA | -0.713 | 0.267 | 0.0256 | Apoptosis, Innate Immune Response, NF-kB, Wnt |
| ATG5-mRNA | -0.75 | 0.282 | 0.026 | Autophagy, Cellular Stress, Innate Immune Response |
| PPP3CA-mRNA | -1.1 | 0.412 | 0.0262 | Adaptive Immune Response, Apoptosis, Growth Factor Signaling, Innate Immune Response, Wnt |
| BRAF-mRNA | -0.408 | 0.159 | 0.03 | Adaptive Immune Response, Angiogenesis, Apoptosis, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, Insulin Signaling, Neurons and Neurotransmission |
| S100A12-mRNA | 1.99 | 0.785 | 0.032 | Innate Immune Response |

| | | | | |
|---|---|---|---|---|
| TNFSF10-mRNA | -1.2 | 0.48 | 0.0337 | Apoptosis, Cytokine Signaling, Innate Immune Response |
| IFNAR1-mRNA | -0.588 | 0.236 | 0.0343 | Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response |
| HMGB1-mRNA | -0.887 | 0.368 | 0.0393 | Apoptosis, Autophagy, Inflammatory Signaling, Innate Immune Response |

## Control vs TBI – Innate immune Response

| Probe Label | Log2 fold change | std error (log2) | P-value | Gene.sets |
|---|---|---|---|---|
| PPP3R1-mRNA | -1.24 | 0.24 | 0.000591 | Adaptive Immune Response, Apoptosis, Growth Factor Signaling, Innate Immune Response, Wnt |
| PIK3CA-mRNA | -0.965 | 0.192 | 0.000704 | Adaptive Immune Response, Angiogenesis, Apoptosis, Autophagy, Carbohydrate Metabolism, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, Insulin Signaling, Lipid Metabolism |
| PIK3R1-mRNA | -1.45 | 0.297 | 0.000868 | Adaptive Immune Response, Angiogenesis, Apoptosis, Autophagy, Carbohydrate Metabolism, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, Insulin Signaling, Lipid Metabolism |
| JAG1-mRNA | -1.47 | 0.304 | 0.000941 | Innate Immune Response, Microglia Function, Notch |
| PIK3CB-mRNA | -0.875 | 0.189 | 0.00122 | Adaptive Immune Response, Angiogenesis, Apoptosis, Autophagy, Carbohydrate Metabolism, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, Insulin Signaling, Lipid Metabolism |
| PPP3CA-mRNA | -1.75 | 0.47 | 0.00474 | Adaptive Immune Response, Apoptosis, Growth Factor Signaling, Innate Immune Response, Wnt |
| BRAF-mRNA | -0.654 | 0.181 | 0.0056 | Adaptive Immune Response, Angiogenesis, Apoptosis, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, Insulin Signaling, Neurons and Neurotransmission |
| MEF2C-mRNA | -1.52 | 0.475 | 0.011 | Growth Factor Signaling, Innate Immune Response, Microglia Function |
| SFTPD-mRNA | -1.95 | 0.641 | 0.0142 | Adaptive Immune Response, Autophagy, Innate Immune Response |
| C1QB-mRNA | 1.6 | 0.528 | 0.0143 | Innate Immune Response |
| MAPK10-mRNA | -1.35 | 0.468 | 0.0181 | Adaptive Immune Response, Apoptosis, Autophagy, Cellular Stress, Growth Factor Signaling, Innate Immune Response, Wnt |
| XIAP-mRNA | -0.871 | 0.304 | 0.0187 | Apoptosis, Innate Immune Response, NF-kB, Wnt |
| PPP3R2-mRNA | -1.88 | 0.668 | 0.0204 | Adaptive Immune Response, Apoptosis, Growth Factor Signaling, Innate Immune Response, Wnt |
| CD14-mRNA | 1.17 | 0.423 | 0.0219 | Apoptosis, Astrocyte Function, Autophagy, Growth Factor Signaling, Innate Immune Response, NF-kB |
| CASP7-mRNA | 1.18 | 0.428 | 0.0223 | Apoptosis, Innate Immune Response |
| HMGB1-mRNA | -1.14 | 0.42 | 0.0234 | Apoptosis, Autophagy, Inflammatory Signaling, Innate Immune Response |
| IRAK1-mRNA | 1.27 | 0.476 | 0.026 | Apoptosis, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, NF-kB |
| ICAM2-mRNA | 0.466 | 0.183 | 0.0311 | Adaptive Immune Response, Innate Immune Response, Matrix Remodeling |
| PILRA-mRNA | 1.23 | 0.491 | 0.0333 | Innate Immune Response |
| BID-mRNA | -0.495 | 0.201 | 0.036 | Apoptosis, DNA Damage, Innate Immune Response |
| PPP3CB-mRNA | -0.717 | 0.293 | 0.0368 | Adaptive Immune Response, Apoptosis, Growth Factor Signaling, Innate Immune Response, Wnt |
| PRF1-mRNA | -2.21 | 0.924 | 0.0402 | Apoptosis, Innate Immune Response |

| | | | | |
|---|---|---|---|---|
| FADD-mRNA | -1.11 | 0.465 | 0.0404 | Apoptosis, Innate Immune Response |
| IFNAR2-mRNA | -0.932 | 0.396 | 0.043 | Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response |
| IFNAR1-mRNA | -0.619 | 0.269 | 0.0467 | Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response |
| TRAF2-mRNA | 1.02 | 0.443 | 0.0472 | Apoptosis, Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response, NF-kB |
| CASP3-mRNA | -0.619 | 0.271 | 0.0485 | Apoptosis, Cellular Stress, DNA Damage, Growth Factor Signaling, Innate Immune Response, Matrix Remodeling |
| PRKDC-mRNA | -1.14 | 0.501 | 0.0486 | Cell Cycle, DNA Damage, Innate Immune Response |

## Control vs AD – Microglia Function

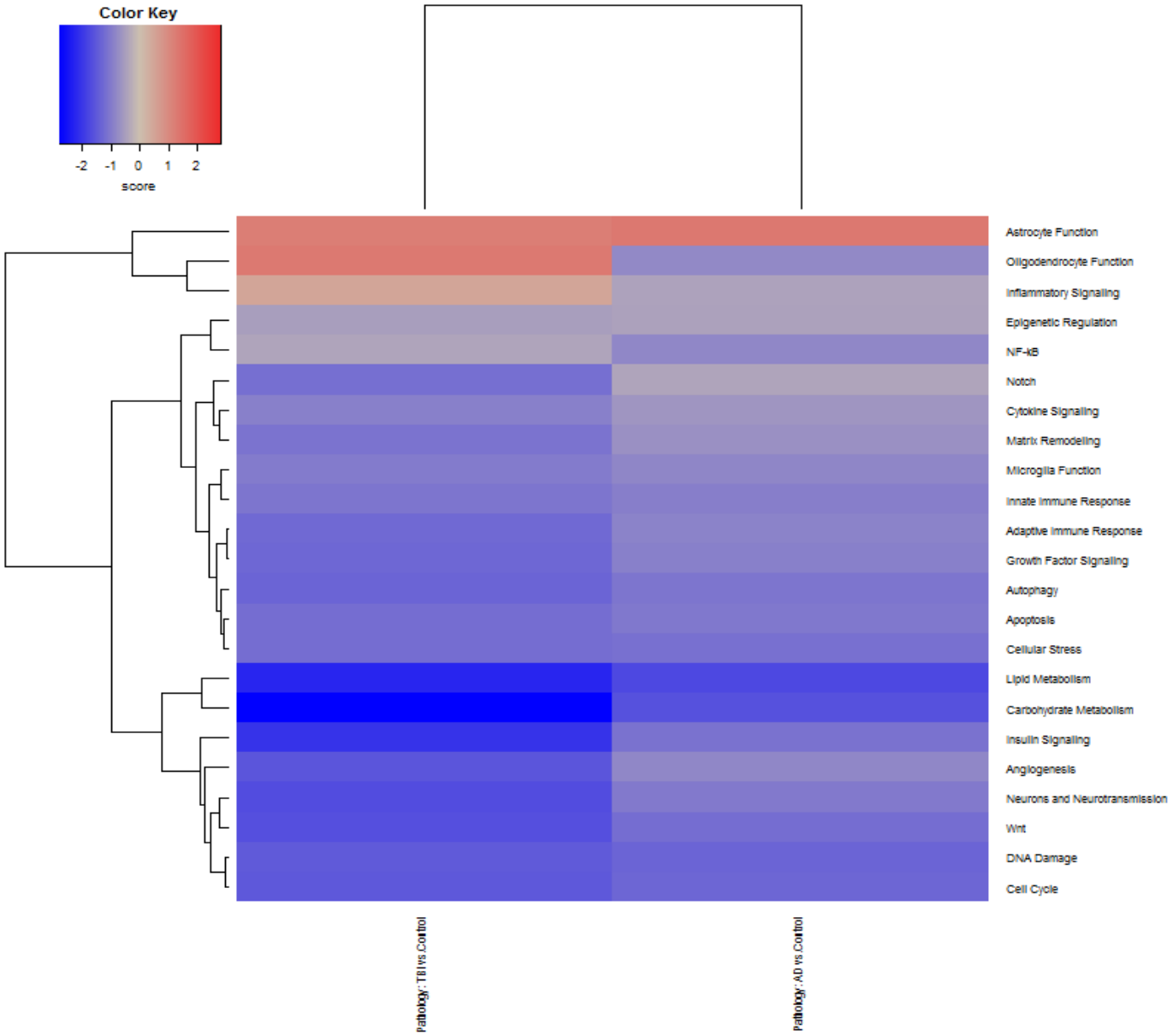
| Probe Label | Log2 fold change | std error (log2) | P-value | Gene.sets |
|---|---|---|---|---|
| CCL4-mRNA | -2.7 | 0.781 | 0.00718 | Cytokine Signaling, Innate Immune Response, Microglia Function, NF-kB |
| FGF13-mRNA | -0.73 | 0.225 | 0.01 | Growth Factor Signaling, Microglia Function |
| TPD52-mRNA | -1.61 | 0.506 | 0.011 | Microglia Function, Neurons and Neurotransmission |
| IL1B-mRNA | -2.23 | 0.702 | 0.0112 | Apoptosis, Cellular Stress, Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response, Microglia Function, NF-kB |
| RPS10-mRNA | -0.762 | 0.244 | 0.0122 | Microglia Function |
| HILPDA-mRNA | 3.38 | 1.11 | 0.0137 | Microglia Function |
| P2RY12-mRNA | -1.78 | 0.59 | 0.0146 | Microglia Function |
| ATP6V1A-mRNA | -0.663 | 0.227 | 0.0169 | Growth Factor Signaling, Insulin Signaling, Microglia Function |
| STMN1-mRNA | -0.68 | 0.241 | 0.0199 | Growth Factor Signaling, Microglia Function |
| PTGER3-mRNA | -1.16 | 0.437 | 0.0265 | Microglia Function, Neurons and Neurotransmission |
| SOD2-mRNA | 2.12 | 0.817 | 0.0287 | Cellular Stress, Microglia Function |
| CCL3-mRNA | -2.47 | 0.959 | 0.0297 | Cytokine Signaling, Microglia Function |
| RPL9-mRNA | -1.01 | 0.404 | 0.0333 | Microglia Function |
| CRIP1-mRNA | -1.12 | 0.447 | 0.0333 | Microglia Function |
| ST3GAL6-mRNA | -0.569 | 0.228 | 0.0343 | Microglia Function, Notch |
| HIST1H1D-mRNA | 1.14 | 0.46 | 0.0349 | Apoptosis, Cellular Stress, Microglia Function |
| RPS21-mRNA | -0.512 | 0.211 | 0.0383 | Microglia Function |
| IGF1-mRNA | -0.645 | 0.278 | 0.0453 | Carbohydrate Metabolism, DNA Damage, Growth Factor Signaling, Insulin Signaling, Microglia Function |
| TMEM64-mRNA | -0.692 | 0.302 | 0.0478 | Microglia Function |

## Control vs TBI – Microglia Function

| Probe Label | Log2 fold change | std error (log2) | P-value | Gene.sets |
|---|---|---|---|---|
| JAG1-mRNA | -1.47 | 0.304 | 0.000941 | Innate Immune Response, Microglia Function, Notch |
| JAM2-mRNA | -1.03 | 0.216 | 0.000997 | Matrix Remodeling, Microglia Function |

| | | | | |
|---|---|---|---|---|
| **FGF13-mRNA** | -1.18 | 0.256 | 0.00126 | Growth Factor Signaling, Microglia Function |
| **TBC1D4-mRNA** | -0.737 | 0.193 | 0.0041 | Microglia Function, Neurons and Neurotransmission |
| **ATP6V1A-mRNA** | -0.894 | 0.258 | 0.00712 | Growth Factor Signaling, Insulin Signaling, Microglia Function |
| **IGF1-mRNA** | -1.06 | 0.322 | 0.00925 | Carbohydrate Metabolism, DNA Damage, Growth Factor Signaling, Insulin Signaling, Microglia Function |
| **STMN1-mRNA** | -0.9 | 0.274 | 0.00953 | Growth Factor Signaling, Microglia Function |
| **ST3GAL6-mRNA** | -0.855 | 0.261 | 0.00957 | Microglia Function, Notch |
| **MEF2C-mRNA** | -1.52 | 0.475 | 0.011 | Growth Factor Signaling, Innate Immune Response, Microglia Function |
| **RPS21-mRNA** | -0.767 | 0.241 | 0.011 | Microglia Function |
| **IGSF10-mRNA** | -2.02 | 0.64 | 0.0117 | Microglia Function |
| **CD83-mRNA** | -0.708 | 0.226 | 0.0121 | Microglia Function |
| **HCAR2-mRNA** | -3.42 | 1.09 | 0.0122 | Microglia Function |
| **RAB6B-mRNA** | -0.58 | 0.186 | 0.0124 | Microglia Function |
| **EEF2K-mRNA** | -1.58 | 0.529 | 0.015 | Growth Factor Signaling, Insulin Signaling, Microglia Function |
| **CD68-mRNA** | 1.95 | 0.681 | 0.0186 | Microglia Function |
| **TLE3-mRNA** | 0.608 | 0.219 | 0.0215 | Microglia Function, Notch, Wnt |
| **CD33-mRNA** | 1.28 | 0.491 | 0.0286 | Adaptive Immune Response, Microglia Function |
| **CRIP1-mRNA** | -1.3 | 0.512 | 0.0319 | Microglia Function |
| **SPP1-mRNA** | 1.78 | 0.724 | 0.0365 | Growth Factor Signaling, Matrix Remodeling, Microglia Function |
| **TMEM100-mRNA** | -1.13 | 0.462 | 0.0374 | Microglia Function |
| **PTMS-mRNA** | 1.41 | 0.589 | 0.04 | Microglia Function |
| **AXL-mRNA** | 0.878 | 0.366 | 0.0401 | Angiogenesis, Autophagy, Microglia Function |
| **LMNB1-mRNA** | -0.487 | 0.204 | 0.0406 | Apoptosis, Cell Cycle, Cellular Stress, Microglia Function |
| **TMEM64-mRNA** | -0.82 | 0.345 | 0.0415 | Microglia Function |
| **RGL1-mRNA** | 0.871 | 0.368 | 0.042 | Growth Factor Signaling, Microglia Function |
| **APOE-mRNA** | 1.42 | 0.601 | 0.0423 | Astrocyte Function, Cellular Stress, Lipid Metabolism, Microglia Function, Neurons and Neurotransmission |
| **LAIR1-mRNA** | 1.23 | 0.524 | 0.0442 | Adaptive Immune Response, Microglia Function |
| **LRRC3-mRNA** | -1.39 | 0.6 | 0.0458 | Microglia Function |
| **LDHA-mRNA** | -1.13 | 0.492 | 0.0468 | Microglia Function |
| **TPD52-mRNA** | -1.32 | 0.576 | 0.0482 | Microglia Function, Neurons and Neurotransmission |

**Heat Map of global directed significance scores (TBI vs Controls; AD vs Controls)**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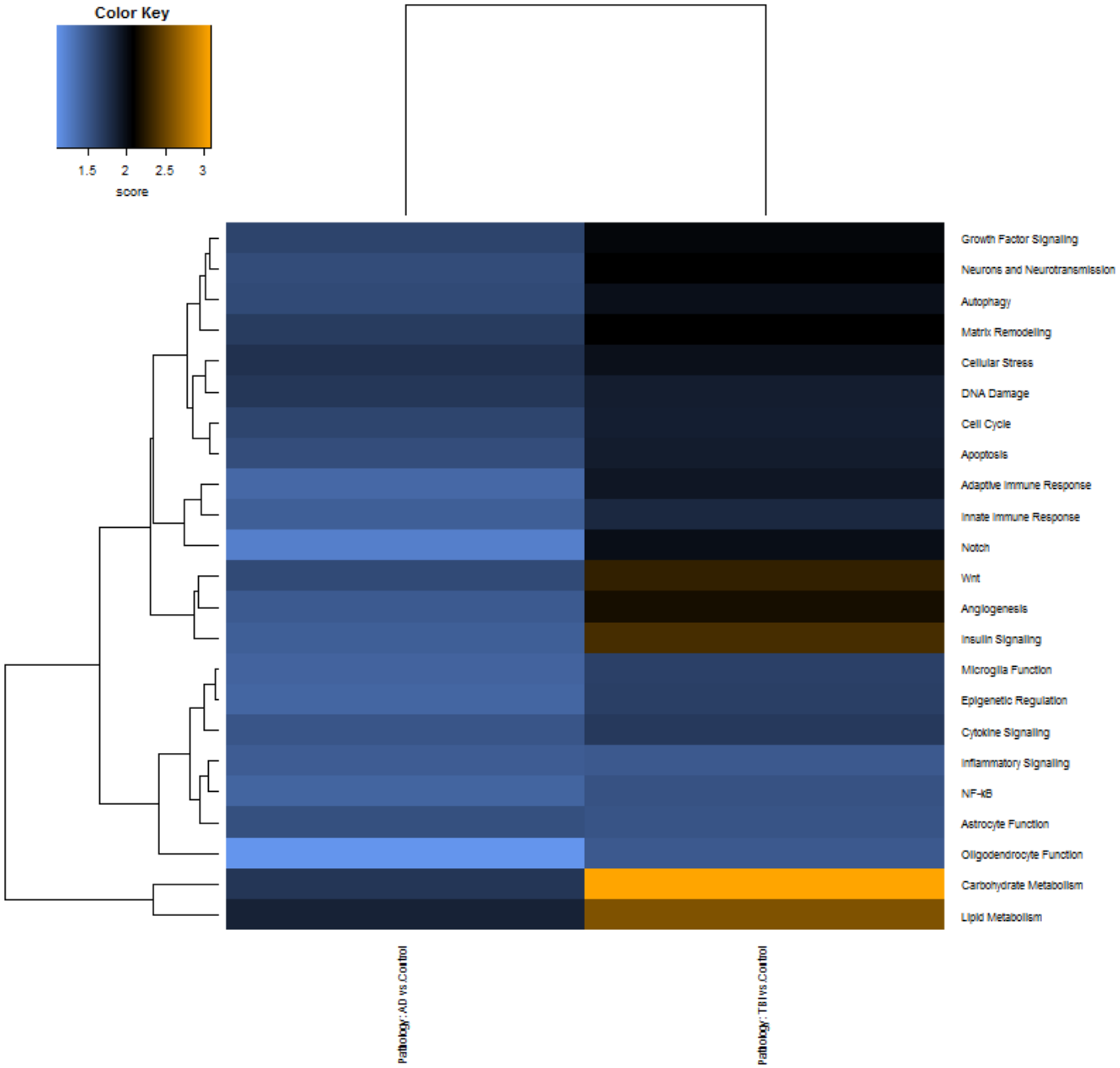
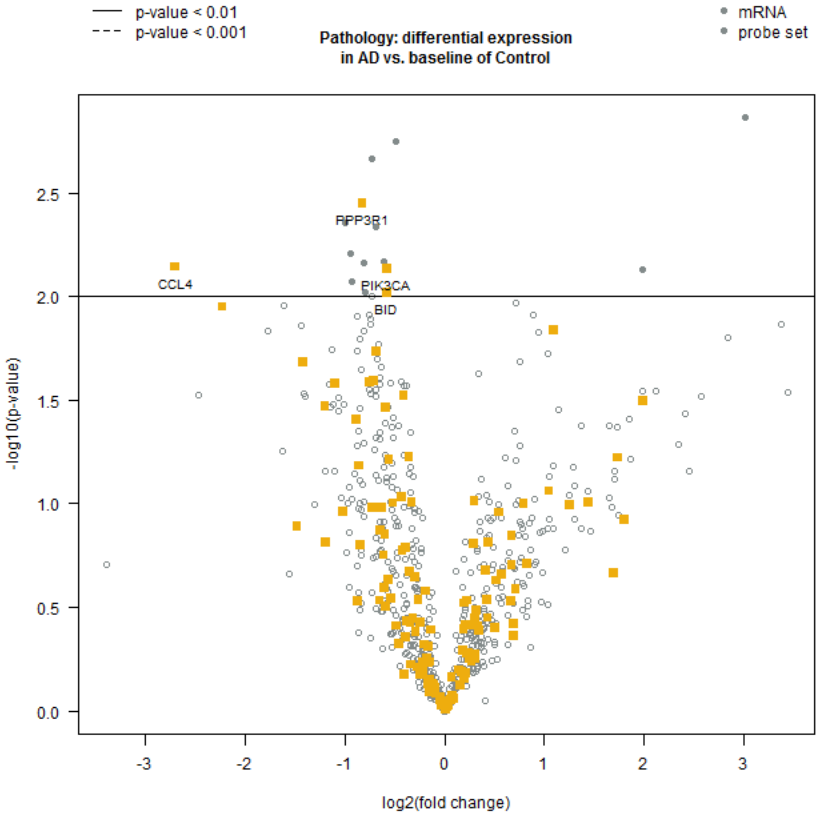
-2
-1
0
1
2
score
Astrocyte Function
Oligodendrocyte Function
Inflammatory Signaling
Epigenetic Regulation
NF-kB
Notch
Cytokine Signaling
Matrix Remodeling
Microglia Function
Innate Immune Response
Adaptive Immune Response
Growth Factor Signaling
Autophagy
Apoptosis
Cellular Stress
Lipid Metabolism
Carbohydrate Metabolism
Insulin Signaling
Angiogenesis
Neurons and Neurotransmission
Wnt
DNA Damage
Cell Cycle
Pathology: TBI vs Control
Pathology: AD vs Control

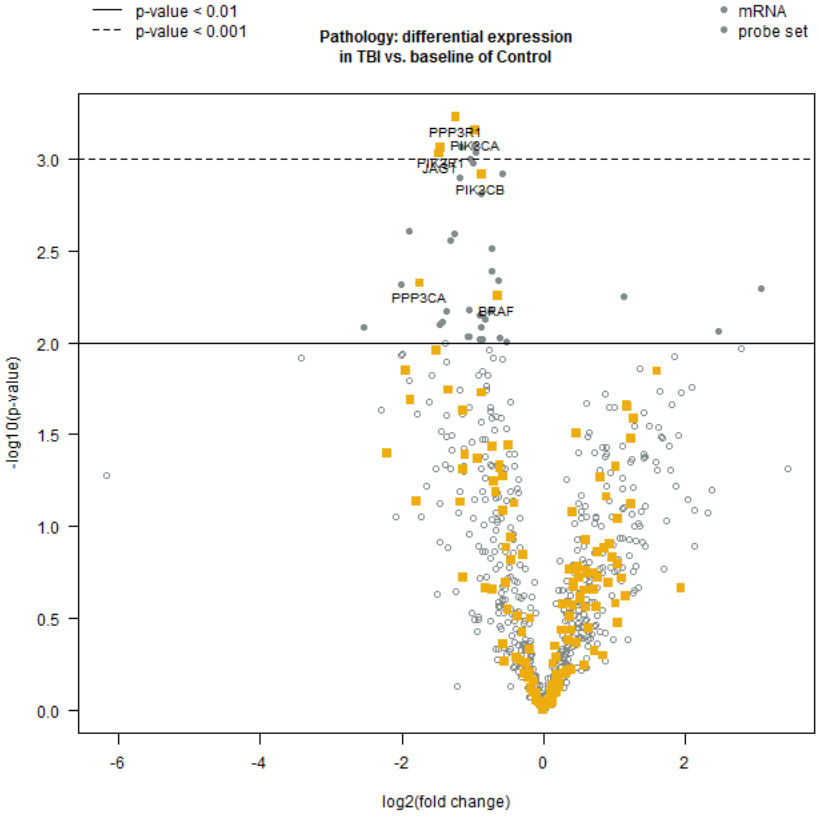
## Heat Map of global directed significance scores (TBI vs Controls; AD vs Controls)



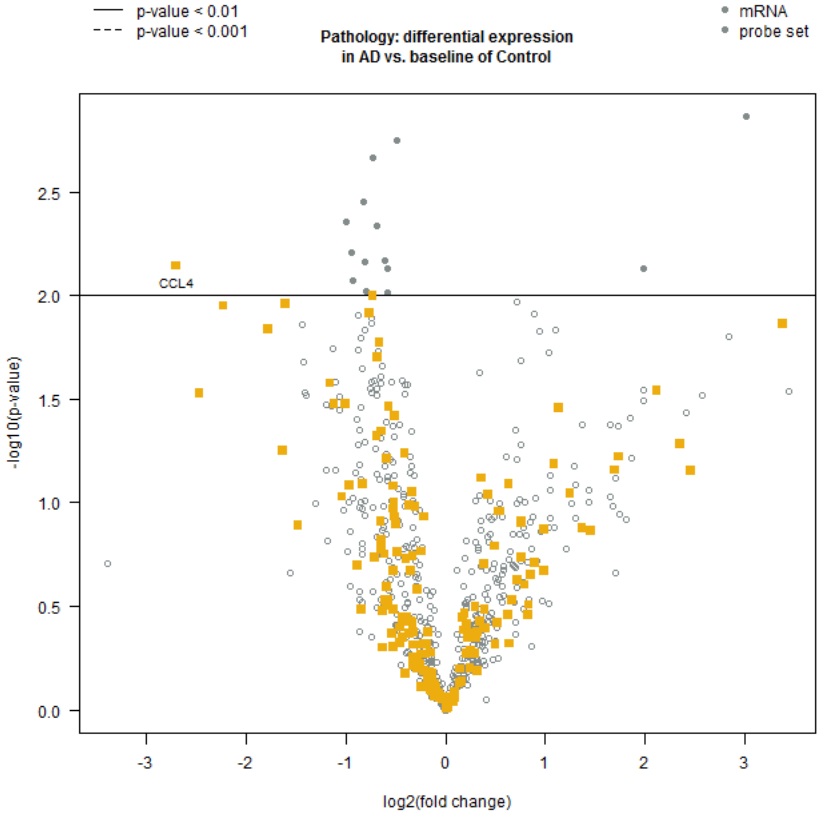
## Volcano Plot – Innate immune response AD vs Controls



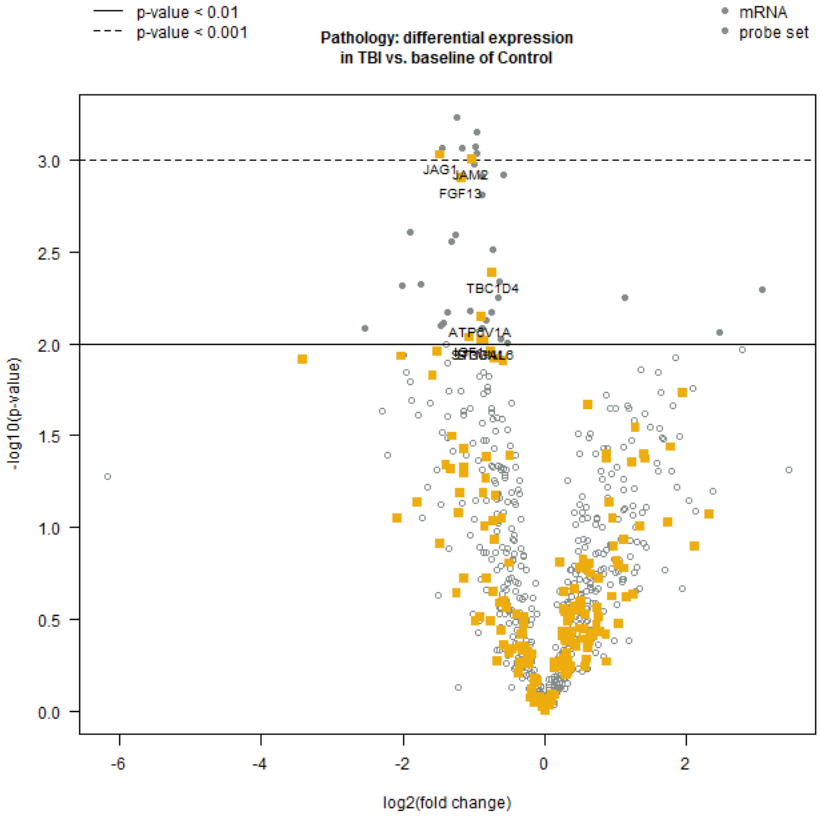

## Volcano Plot – Innate immune response TBI vs Controls



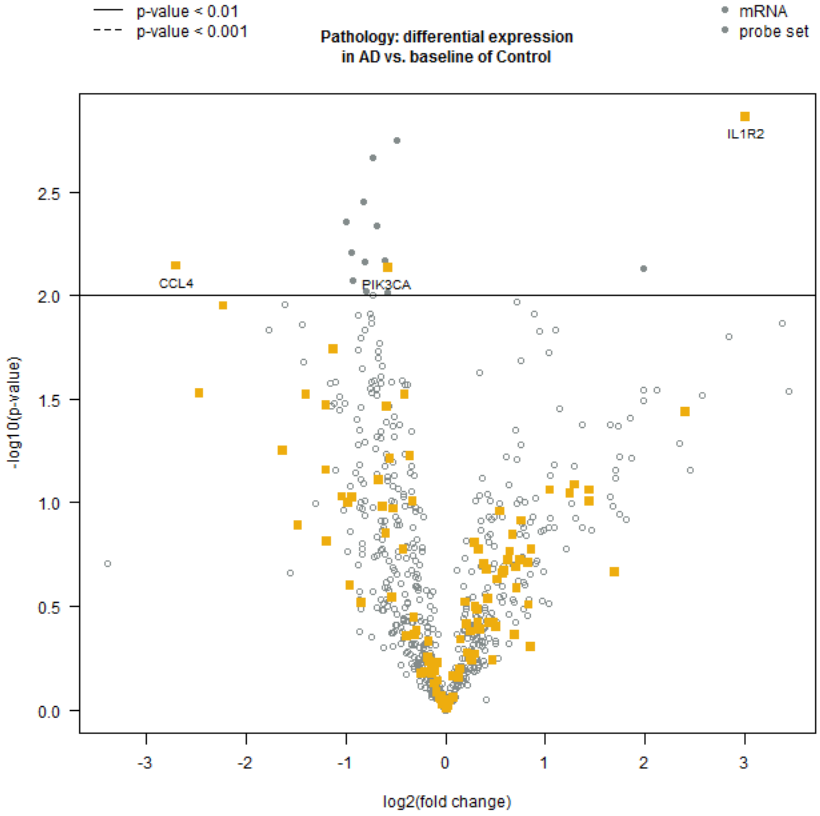
## Volcano Plot – Microglia Function AD vs Controls



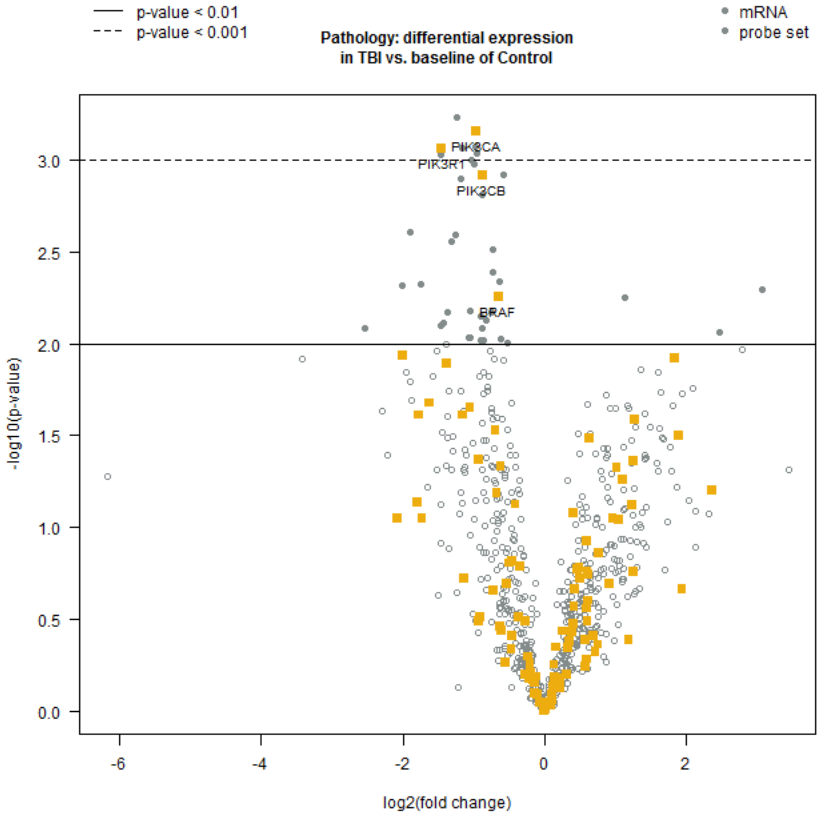

## Volcano Plot – Microglia Function TBI vs Controls



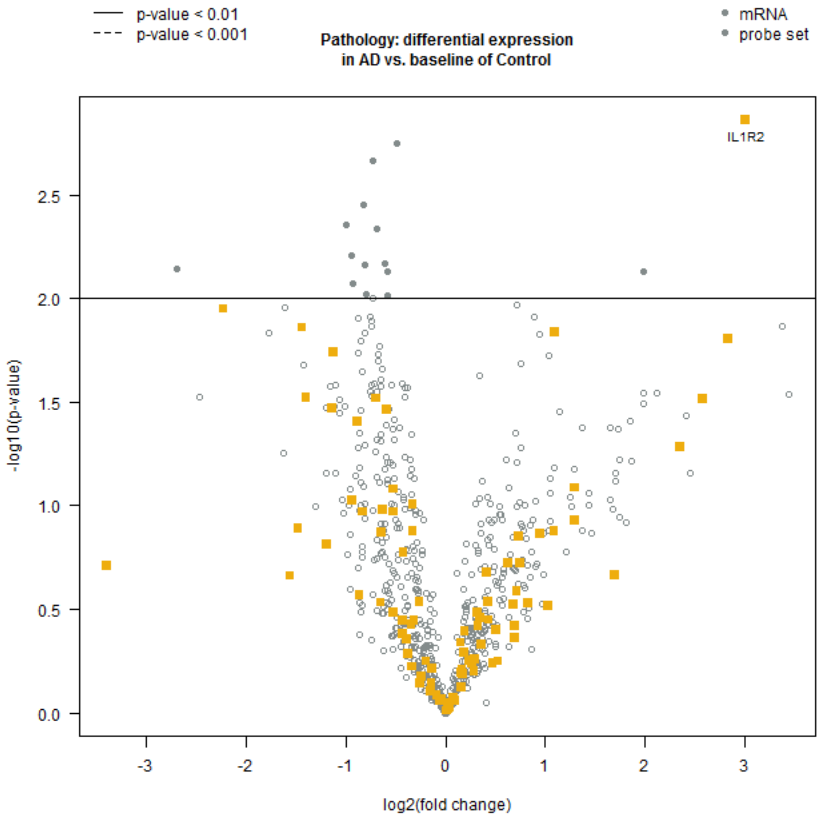
## Volcano Plot – Cytokine signaling AD vs Controls



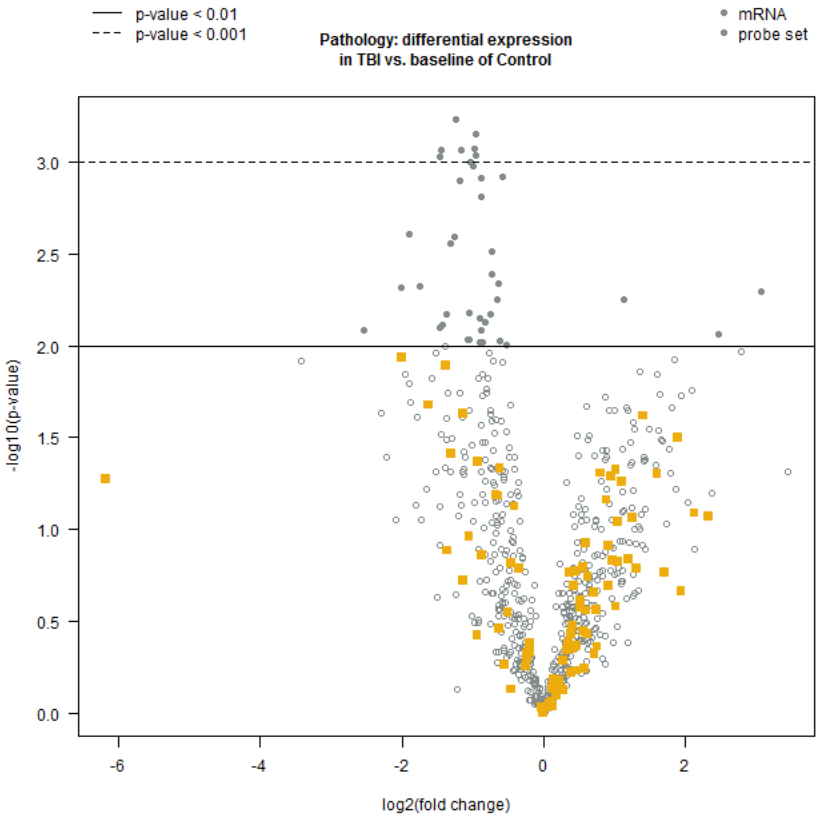

## Volcano Plot – Cytokine signaling TBI vs Controls



## Volcano Plot – Inflammatory Signaling (AD vs Controls)




## Volcano Plot – Inflammatory Signaling (TBI vs Controls)




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

Nothing to Report

**How were the results disseminated to communities of interest?** Nothing to report

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

We will continue studies in subtask 2-8 of Major Task 1 over our staggered schedule. We aim to have completed all human histological analyses, including single cell analyses of autopsy tissue through our subcontract with Barrow Institute.

**Subtask 2**: **Complete** breeding of cohorts for studies.
**Subtask 3**: **Complete** histopathological analyses of microglia pathobiology in human AD/CTE cases (n=80; 10/group with 8 different groups).
**Subtask 4**: **Continue** administering injuries to remaining uninjured Young cohort (36 per genotype). Mice used will be mixed gender WT, hTau, and $APP^{NLF}$ transgenic mice on the C57BL/6 background, aged 3 months at injury. For each of the three genotypes there will be two groups - r-mTBI young and r-sham young - each with 18 mice per group. Of these 18 mice, 6 will be euthanized for analysis at each of the three timepoints
**Subtask 5**: **Begin** euthanasia of Young cohort; for each of the three genotypes (WT, hTau, and $APP^{NLF}$) there will be 36 mice, of which 18 will have received r-mTBI and 18 received r-sham. For each genotype, 6 r-mTBI and 6 r-sham mice will be euthanized at 1 day post-injury, 3 months post-injury and 9 months post-injury.
**Subtask 6: Begin** sectioning and histopathological staining of Young cohort tissues with microglia, tau and amyloid antibodies; and TUNEL, BrdU, EM preps.

**Subtask 7: Begin** Stereological analyses and Image quantitation of brain sections from Young cohort (staggered over time)
**Subtask 8: Continue** Administering injuries to Aged cohort (36 per genotype).
Mice used will be mixed gender WT, hTau, and $APP^{NLF}$ transgenic mice on the C57BL/6 background, aged 12 months at injury.

**MAJOR TASK 2**
**Subtask 1: Continue** Laser capture microdissection of IBA1/CD68+ microglia from human AD/CTE cases for single cell array for gene expression profiling.
**This will be conducted at Barrow Neuroscience Institute**.

4. **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

5. **CHANGES/PROBLEMS:**

**Changes in approach and reasons for change**

There is no change in our approach.

**Actual or anticipated problems or delays and actions or plans to resolve them**

Obtaining adequate numbers of human autopsy brain tissue for our neuropathological analyses:
We have applied to the NIH biobank to conduct studies on autopsy tissue. We have received 52 samples out of 80 requested. 16 samples will be sent to us over the next month from Harvard BioBank. We are also waiting for samples from Boston BioBank, however this is not guaranteed from the same brain region. We will consider approaching other brain banks with repetitive TBI history to complete the cohort for our TBI autopsy cases.

Our ACURO protocol was accepted in mid November 2018: We are 3 months behind our initial schedule for the animal studies. We will try to stagger more mice to prevent a significant lag in time for our planned studies. We may also consider reducing our longest time point from 9 to 6 months post-injury to accommodate our schedule within the 3 years.

Apart from these set backs we do not anticipate any new problems going forward.

**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:** Nothing to Report

**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

**Publications, conference papers, and presentations**

N/A

**Journal publications.** Nothing to Report

**Books or other non-periodical, one-time publications.** Nothing to Report

**Other publications, conference papers, and presentations.** *Nothing to Report*

**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*

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

| Personnel | Role | Percent Effort |
|---|---|---|
| Dr. Fiona Crawford. | Principal Investigator | 5% |
| Dr. Joseph Ojo | Co-Principal Investigator | 20% |
| Dr. Benoit Mouzon | Co-Investigator – TBI animal models | 5% |

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?** None

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

## 8. SPECIAL REPORTING REQUIREMENTS

**COLLABORATIVE AWARDS:** *N/A*

**QUAD CHARTS:** *(Attached)*

## 9. APPENDICES – Nothing to report

# Glial cell dysfunction in the pathobiological sequelae of repetitive mild traumatic injury

Log Number AZ170115

W81XWH-18-1-0811

**PI:** Drs Fiona Crawford / Joseph Ojo **Org:** The Roskamp Institute, Sarasota, FL **Award Amount:** $703,488,80

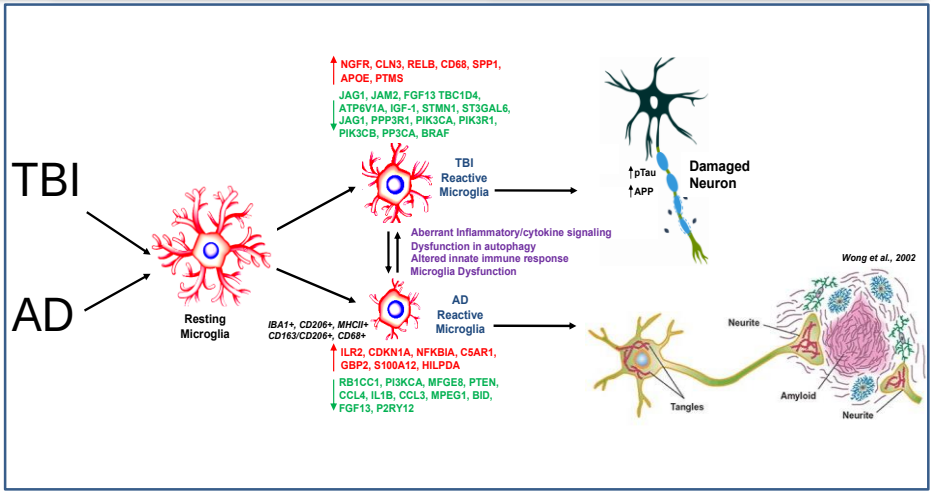
**Study Aim 1:** Delineation of the effects of chronic repetitive mTBI on microglia pathobiology and related proteinopathy in tau and amyloid bearing preclinical models at multiple time points post-injury, and validation in autopsied brains from human AD/CTE cases.

**Study Aim 2:** Generation of single cell genomic profiles in different populations of microglia obtained from preclinical models and validation in autopsied brains from human AD/CTE cases.

### Approach

i) Histopathological assessment of microglia pathobiology in WT, hTau and APPNLF mouse models exposed to r-mTBI, or sham injury, when young (3 months) or aged (12 months) and analyzed at 24hrs, 3 and 9 months post-injury. Histopathological assessment of microglia pathobiology and in human AD/TBI cases.

ii) Single cell genomic profiles in different populations of microglia obtained from young and aged WT, hTau, and APPNLF mice exposed to r-mTBI or r-sham and analyzed at 24hrs, 3 and 9 months post-injury. Validation of distinct microglial cell population of interest and identified gene profiles using flow cytometry and cell sorting. Single cell genomic profiles of microglia from autopsied human AD/CTE brains.




**Accomplishment**

Completed interrogation of human control, AD and a fraction of the cohort of TBI autopsy brain tissue for histopathological assessment of IBA1, MHCII, CD68, CD45, CD206, CD163, PHF1, and 4G8. We have also generated gene analyses of microglia from control, TBI and AD.

## Timeline and Cost

| Activities CY | 19 | 20 | 21 |
|---|---|---|---|
| MAJOR TASK ONE OR AIM 1 | | | |
| MAJOR TASK TWO OR AIM 2 | | | |
| **Estimated Direct Budget (500K)** | **$130K** | **$205K** | **$165K** |

**Last updated:** (September 2018)

### Goals/Milestones

**CY18 Goal**

☑ Obtain regulatory approval to begin animal and human specimen studies

☑ Histological assessment of human control/TBI/AD brains tissue

**CY19 Goals**

☑ Initiate breeding and administer injuries to to different mouse models

☑ Continue histological assessment of human control/TBI/AD brains tissue

☑ Gene analyses of microdissected microglia from autopsy brain tissue

**CY20 Goals**

☑ Histological assessment of microglia and proteinopathy in mouse models

☑ Complete human histological and gene array analyses

**CY21 Goal**

☑ Gene analyses of microdissected microglia from mouse models

☑ Validation experiments of gene array data in mouse models

**Comments/Challenges/Issues/Concerns**

- Waiting for more human tissue from TBI cases to complete TBI cohort
- **Budget Expenditure to Date**

Projected Expenditure: $188,369 (Directs + Indirects)

Actual Expenditure: $249.513.18 (Directs + Indirects)