A comparative study of Traumatic Brain Injury and Familial Alzheimer's Disease

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Abstract:
Microglia, the resident macrophage in the central nervous system, can evolve into disease-associated microglia, a specific subtype that is seen across multiple neurodegenerative diseases. In order to further understand microglial states in neurodegeneration, we compared the transcriptional programs of microglia following traumatic brain injury and in Alzheimer's disease using published single-cell RNA sequencing datasets. We discovered that, while there is not a significant overlap between the microglial populations in the two datasets, there are some overlapping genes of interest that implicate disease-associated microglia.

Introduction
Microglia, the resident macrophage in the central nervous system, play an important role in the immune system and, more specifically, in neuroinflammation ((D.J. DiSabato, N. Quan, J.P. Godbout. Neuroinflammation: the devil is in the details. *J Neurochem*. **139**, 136-153 (2016).)). In their healthy state, microglia phagocytose debris. However, prolonged stimulation might activate microglia, causing them to switch to a proinflammatory phenotype that might be harmful to neurons ((Y. S. Kim, T. H. Joh. Microglia, major player in the brain inflammation: their roles in the pathogenesis of Parkinson's disease. *Experimental & molecular medicine*, **38**, 333–347 (2006).)). Two conditions, traumatic brain injury (TBI) and Alzheimer's Disease (AD), adopt this inflammatory state of microglia ((F. Leng, P. Edison. Neuroinflammation and microglial activation in Alzheimer's Disease: Where do we go from here? *Nature Reviews Neurology*, **17**, 157–172 (2020).)).

Alzheimer's disease (AD) is a neurodegenerative disease with progressive neuronal loss. AD is prevalent in 1 in 10 Americans over 65 years old and contributes to over 100,000 deaths a year ((Alzheimer's Association. 2021 Alzheimer's Disease facts and Figures.https://www.alz.org/alzheimers-dementia/facts-figures#:~:text=More%20than%206%20million%20Americans%20of%20all%20ages%20have%20Alzheimer's,older%20(10.7%25)%20has%20Alzheimer's (2021).)). Familial AD, a subtype of AD, which only makes up around 1%, is detrimental to families. However, it offers a path to studying sporadic AD due to identical pathological features ((C. Arber, J. Toombs, C. Lovejoy, N. S. Ryan, R. W. Paterson, N. Willumsen, E. Gkanatsiou, E. Portelius, K. Blennow, A. Heslegrave, J. M. Schott, J. Hardy, T. Lashley, N. C. Fox, H. Zetterberg, S. Wray. Familial alzheimer's disease patient-derived neurons reveal distinct mutation-specific effects on amyloid beta. *Molecular Psychiatry*, **25**, 2919–2931 (2019).)). Two features of AD are amyloid beta plaques and neurofibrillary tangles. Prolonged inflammation caused by the two features has been identified to be harmful to neurons ((F. Leng, P. Edison. Neuroinflammation and microglial activation in Alzheimer's Disease: Where do we go from here? *Nature Reviews Neurology*, **17**, 157–

172 (2020).)), ((S. Bachiller, I. Jiménez-Ferrer, A. Paulus, Y. Yang, M. Swanberg, T. Deierborg, A. Boza-Serrano. Microglia in neurological diseases: A road map to brain-disease dependent-inflammatory response. *Frontiers in Cellular Neuroscience*, **12** (2018).)). Such results were confirmed in a post-mortem study comparing young patients experiencing systemic inflammation and people with dementia, as well as in genome-wide association studies (GWAS) ((F. Leng, P. Edison. Neuroinflammation and microglial activation in Alzheimer's Disease: Where do we go from here? *Nature Reviews Neurology*, **17**, 157–172 (2020).)), ((V. Landel, K. Baranger, I. Virard, B. Loriod, M. Khrestchatisky, S. Rivera, P. Benech, F. Féron. Temporal gene profiling of the 5XFAD transgenic mouse model highlights the importance of microglial activation in Alzheimer's disease. *Molecular neurodegeneration*, **9** (2014).)). More interestingly, the result of GWAS suggests that neuroinflammation might play an initiating role in AD but could later induce further neuronal damage as there is a negative correlation between inflammation and the structure and function of the brain ((F. Leng, P. Edison. Neuroinflammation and microglial activation in Alzheimer's Disease: Where do we go from here? *Nature Reviews Neurology*, **17**, 157–172 (2020).)).

On the other hand, in the United States, 1.7 million people suffer from Traumatic Brain Injury (TBI) per year, with common encounters of concussion, extra-axial hematomas, contusion, et cetera ((A. Georges, J.M. Das. Traumatic Brain Injury. *StatPearls [Internet]*. (2022).)). Common effects include lifelong motor, cognitive, and behavioral disabilities resulting from neuron death and are observed both directly from trauma and secondary pathways ((P. Kermer, N. Klöcker, M. Bähr. Neuronal death after brain injury. Models, mechanisms, and therapeutic strategies in vivo. *Cell and tissue research*, **298**, 383–395 (1999).)). Though primary injuries, injury from the impact, are unpreventable, secondary injuries, the subsequent damage, are potentially intervenable. One important response to TBI identified in previous studies is neuroinflammation. Acute inflammation is thought to help sterilize the wound and help with initial repair, but inflammation may induce secondary injury with neurotoxicity and further brain damage ((K. Somebang, J. Rudolph, I. Imhof, L. Li, E. C. Niemi, J. Shigenaga, H. Tran, T. M. Gill, I. Lo, B. A. Zabel, G. Schmajuk, B. T. Wipke, S. Gyoneva, L. Jandreski, M. Craft, G. Benedetto, E. D. Plowey, I. Charo, J. Campbell, C. J. Ye, S. S. Panter, M.C. Nakamura, W. Eckalbar, C. L. Hsieh. Ccr2 deficiency alters activation of microglia subsets in Traumatic Brain Injury. *Cell Reports*, **36**, 109727 (2021).)). Such a profile seems strongly similar to the immune mechanisms of inflammation in Alzheimer’s Disease. Further, studies have indicated that TBI increases the risk of the potential development of AD ((F. Leng, P. Edison. Neuroinflammation and microglial activation in Alzheimer's Disease: Where do we go from here? *Nature Reviews Neurology*, **17**, 157–172 (2020).)).

In order to further understand the role of inflammation on neurodegeneration and the connection between the two diseases, a comparison of the characteristics of neuroinflammation of AD and TBI needs to be established. Previous studies have identified Ccr2 as a damaging neuroinflammatory gene in TBI, while the ligand of Ccr2, Ccl2 was also previously shown to be highly upregulated in the 5xFAD model ((K. Somebang, J. Rudolph, I. Imhof, L. Li, E. C. Niemi, J. Shigenaga, H. Tran, T. M. Gill, I. Lo, B. A. Zabel, G. Schmajuk, B. T. Wipke, S. Gyoneva, L. Jandreski, M. Craft, G.

Benedetto, E. D. Plowey, I. Charo, J. Campbell, C. J. Ye, S. S. Panter, M.C. Nakamura, W. Eckalbar, C. L. Hsieh. Ccr2 deficiency alters activation of microglia subsets in Traumatic Brain Injury. *Cell Reports*, **36**, 109727 (2021).)). However, there is currently no literature establishing an overall comparison between TBI and AD in the microglia transcriptional profile. Here, we compare single-nuclei or single-cell sequencing data from TBI and 5xFAD mice. We find that both TBI and FAD contain markers of highly activated microglia and despite the differences, inflammation seems to play a large role in its gene signature.

Results

Microglia derived from TBI and FAD do not share overall transcriptional identities

In order to understand the similarities and differences between microglia in TBI and AD, we analyzed transcriptomic data collected from microglia following TBI and microglia from FAD mice ((K. Somebang, J. Rudolph, I. Imhof, L. Li, E. C. Niemi, J. Shigenaga, H. Tran, T. M. Gill, I. Lo, B. A. Zabel, G. Schmajuk, B. T. Wipke, S. Gyoneva, L. Jandreski, M. Craft, G. Benedetto, E. D. Plowey, I. Charo, J. Campbell, C. J. Ye, S. S. Panter, M.C. Nakamura, W. Eckalbar, C. L. Hsieh. Ccr2 deficiency alters activation of microglia subsets in Traumatic Brain Injury. *Cell Reports*, **36**, 109727 (2021).)), ((Y. Zhou, W.M. Song, P.S. Andhey, A.Swain, T. Levy, K. Miller, P. Poliani, M. Cominelli, S. Grover, S. Gilfillan, M. Cella, T. Ulland, K. Zaitsev, A. Miyashita, T. Ikeuchi, M. Sainouchi, A. Kakita, D.A. Bennett, J.A. Schneider, M.R. Nicholas, S.A. Beausoleil, J.D. Ulrich, D.M. Holtzman, M.N. Artyomov, M. Colonna. Human and mouse single-nucleus transcriptomics reveal TREM2-dependent and TREM2-independent cellular responses in Alzheimer's disease. *Nat Med,* **26**,131-142 (2020).)) Zhou et al. collected nuclei from the brains of 7-month-old male and female 5XFAD and WT mice for sequencing via snRNA-seq. Meanwhile, Hsieh et al. administered a controlled cortical impact TBI to male mice at 3-4 months of age and isolated leukocytes from the brains 4 days following TBI for scRNAseq. Because Zhou et al. collected all nuclei, while Hsieh et al. only collected leukocytes, we subsetted the FAD samples for leukocytes by using common gene features (Trem2, Csf1r). First, we integrated TBI with Sham and FAD with WT to identify specific markers for each condition. We identified 17 clusters for TBI vs Sham (Figure 1 B) and 15 for FAD vs WT (Figure 1 B).

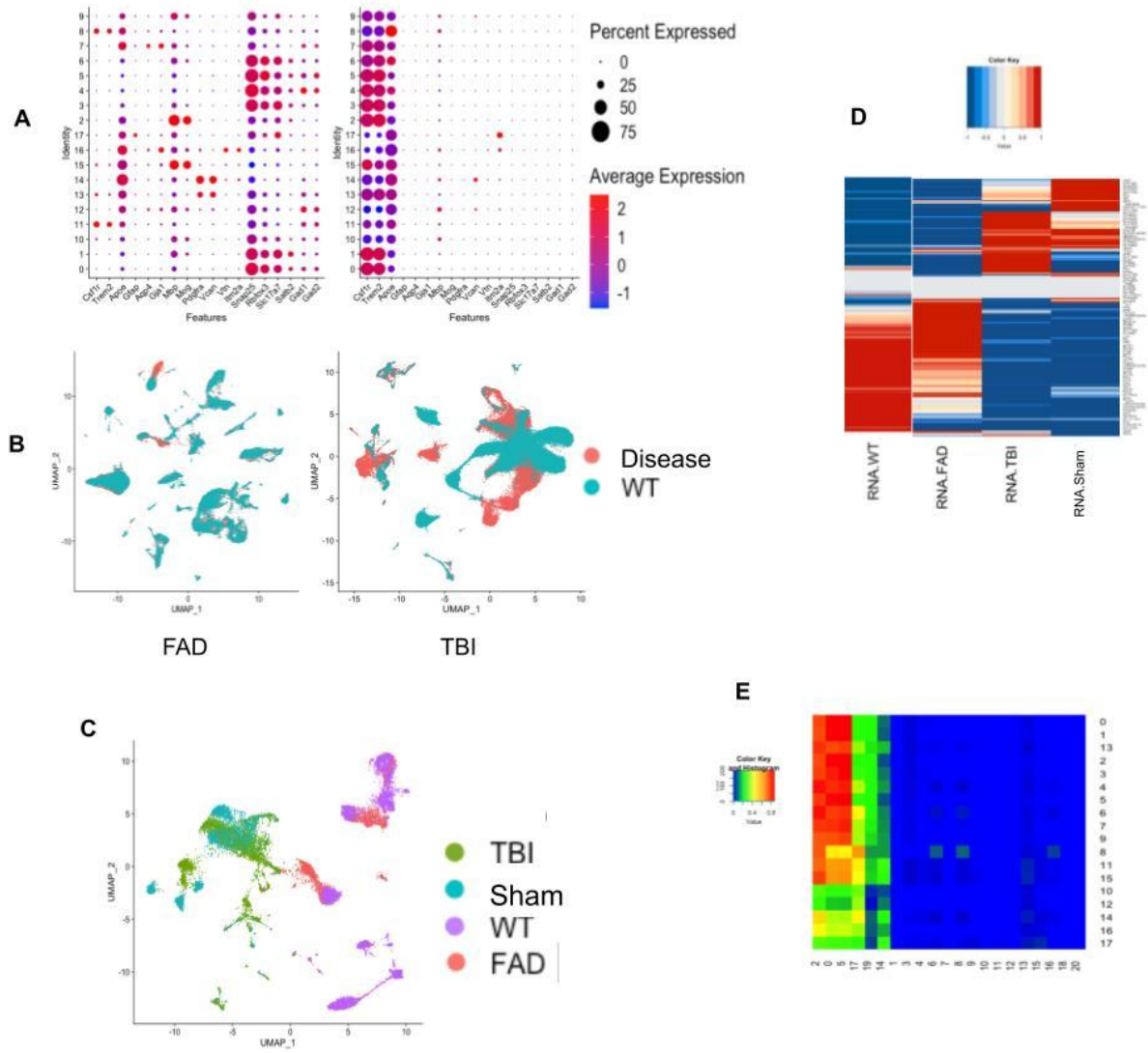


Figure 1: FAD and TBI microglia do not transcriptionally overlap
(A） Dot plot of TBI and FAD datasets (Csf1r, Trem2, Apoe, Gfap, Aqp4, Gja, Mbp, Mog, Pdgfra, Vcan, Vtn, Itm2a, Snap25, Rbfox3, Slc17a7, Satb2, Gad1, Gad2). (B) UMAPs of microglia subsetted by expression levels of Csf1r and Trem2 from the FAD and TBI datasets. (C) UMAP of FAD and TBI microglia integrated. (D) Heatmap showing top markers for each disease condition. (E) Correlation plot between FAD (Column) and TBI (Row) clusters.

In order to understand the similarities of these cell types, we integrated microglia from all of the datasets (TBI, Sham, FAD, WT). We observed that there was minimal overlap between the datasets, and thus the characteristics of the microglia isolated in these datasets are significantly different (Figure 1 C). When looking at the average expression across genotypes, we similarly observed a few differentially expressed genes overlapping between TBI and FAD (Figure 1 D). In order to directly explore the overlap between clusters in the two datasets, we correlated gene expression in each cluster and

observed that all clusters in the TBI dataset correlate with 6 clusters from the FAD dataset, while the remaining 15 clusters from the FAD dataset are not found in the TBI dataset (Figure 1 E).

A potential explanation for the discrepancy between TBI and FAD might be due to the single cell vs single nucleus sequencing difference where some genes might be expressed differently in the cytoplasm and the nucleus. Further, the age difference, FAD mice being 7 months and TBI being 3-4 months might further cause differences in gene expression induced by normal aging. Lastly, the difference in FAD being mixed male and female while TBI being male might also cause differences in gene expression. These changes were evidenced through the drastic differences between WT and Sham which supposedly should be the same.

Genes involved in the inflammatory response are shared between TBI and FAD microglia

In order to understand how functionally the microglia in TBI samples and FAD are similar and different, we utilized Gene Ontology analysis. Based on the markers of TBI and FAD identified, we did a Venn diagram of top markers for TBI and for FAD microglia compared to their controls with avg_log2FC>0. We found 4 overlapping upregulated genes of TBI and FAD (Cst7, Lyz2, Lpl, Apoe) (Figure 2). Lpl correlates with microglia reactivity while Cst7, Lyz2, and Apoe are disease-associated microglia genes observed in multiple neurodegenerative diseases ((A. Grubman, X. Y. Choo, G. Chew, J. F. Ouyang, G. Sun, N. P. Croft, F. J. Rossello, R. Simmons, S. Buckberry, D. V. Landin, J. Pfluege, T. H. Vandekolk, Z. Abay, Y. Zhou, X. Liu, J. Chen, M. Larcombe, J. M. Haynes, C. McLean, S. Williams, S.Y. Chai, T. Wilson, R. Lister, C.W. Pouton, A.W. Purcell, O.J.L. Rackham, E. Petretto, J. M. Polo. Transcriptional signature in microglia associated with AB plaque phagocytosis. Nature Communications, **12**, 3015 (2021))), ((B. A. Loving, M. Tang, M. C. Neal, S. Gorkhali, R. Murphy, R. H. Eckel, K. D. Bruce. Lipoprotein Lipase Regulates Microglial Lipid Droplet Accumulation. *Cells*, **10**, 198. (2021))). More interestingly, Lpl and Cst7 are exclusively expressed in highly activated microglia, a subtype of microglia that triggers immuno-inflammatory responses in the aged brain, while Apoe and Lyz2 are both potential markers of highly activated microglia but are also expressed in other subclusters of microglia ((C. Jin, Y. Shao, X. Zhang, J. Xiang, R. Zhang, Z. Sun, S. Mei, J.Zhou, J. Zhang, L. Shi. A Unique Type of Highly-Activated Microglia Evoking Brain Inflammation via Mif/Cd74 Signaling Axis in Aged Mice. *Aging and disease*, **12**, 2125–2139 (2021).)). The functionality of these genes was mapped and indicated that they all regulated inflammatory responses, consistent with previous literature. These data show that, while the TBI and AD datasets do not overlap significantly, there is an overlap in the transcriptional states of the microglia.

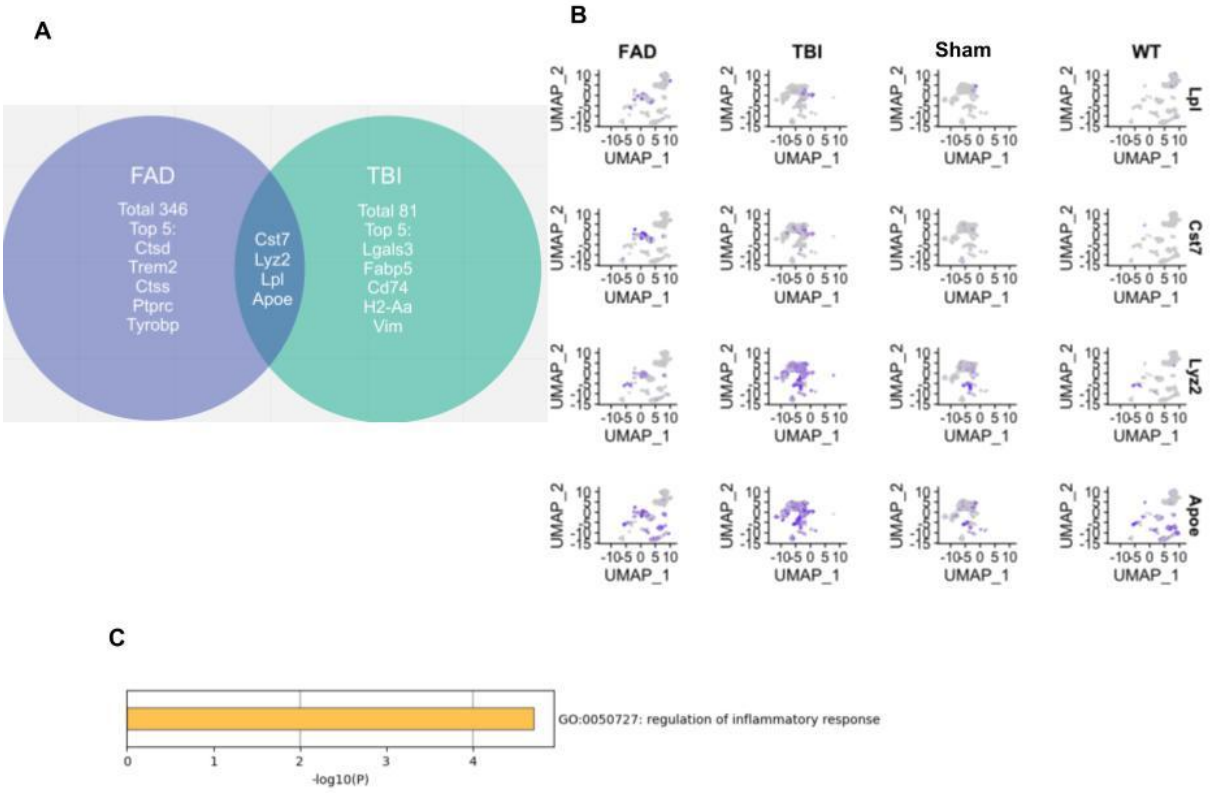


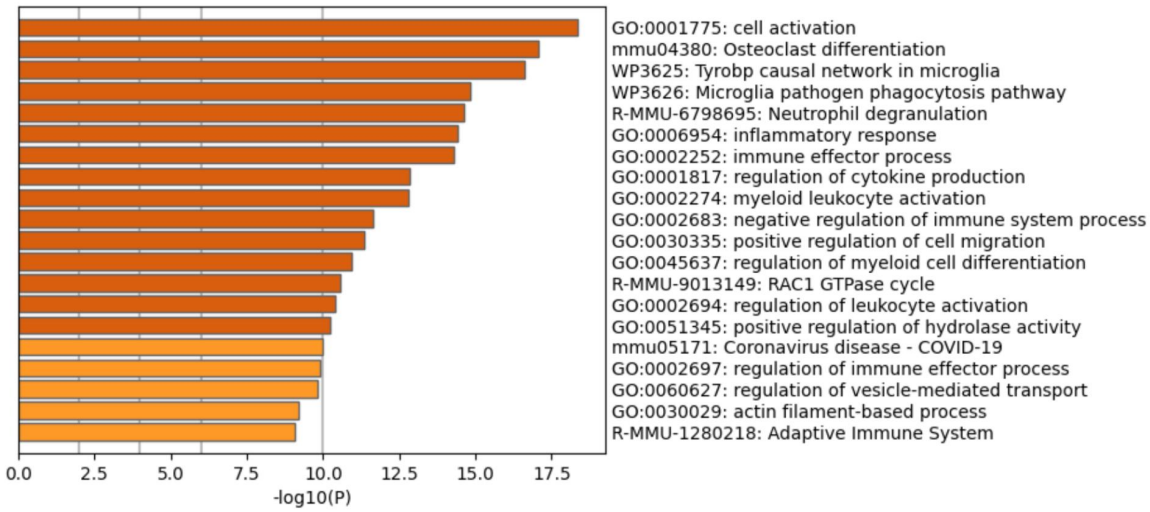
Figure 2: There are four shared markers of TBI and FAD

Venn diagram and GO term for overlapping regions. Upregulated genes were selected with the criteria of avg_log2FC>0 for both TBI and FAD.

Different genes with similar functions underlie the distinction between microglia in TBI and FAD

We further looked into the functionality of upregulated genes that are different between TBI and FAD in the leukocyte population by performing GO analyses on differentially expressed genes. TBI and FAD shared the GO terms “Neutrophil degranulation” and “regulation of leukocyte activation”. There was very little overlap in the specific terms yet both samples preserve a similar identity on a broader scale (Figure 3). The majority of the terms are focused on cytokine and inflammation. This shows that despite the major differences observed in the UMAP, the functionality of the upregulated genes is similar. However, the precise reason for such discrepancy needs to be further investigated.

A



B

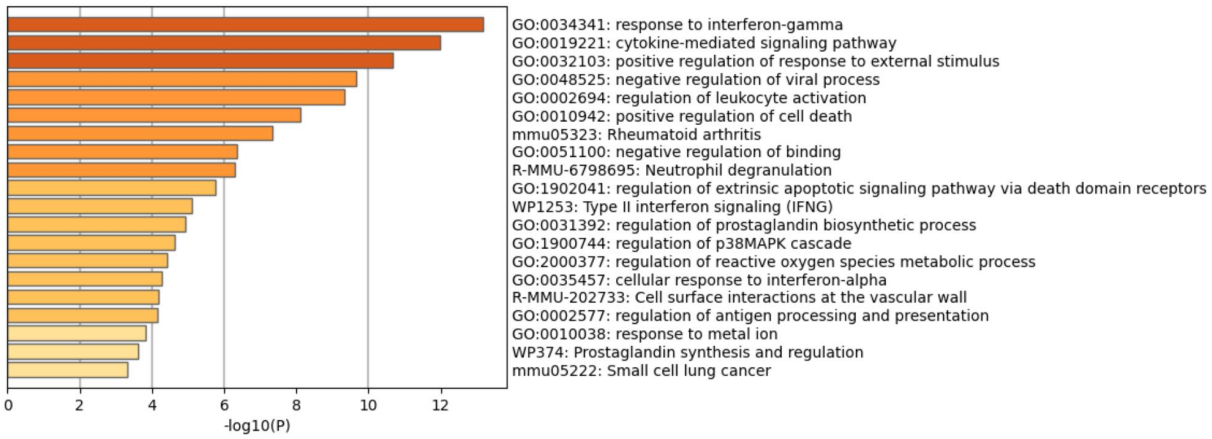


Figure 3 GO terms of unique upregulated genes in TBI and FAD
GO terms of upregulated genes in FAD (A) and TBI (B) that are not shared between genotypes.

Discussion

In order to further understand the role of inflammation, we compared published single-cell RNA sequencing datasets from the 5xFAD mouse model of Alzheimer's disease to a controlled cortical impact model of TBI and their respective controls. We showed that the microglia signature is relatively different between TBI and FAD as shown through the UMAP (Figure 1). However, there are fundamental differences between the studies from which these data are derived, such as the age and sex of the mice, which might cause an inherent mismatch in gene expression. Further, the sequencing method also differs which might contribute to the difference: 5xFAD is sequenced through single-nuclei sequencing while the TBI model is sequenced through single-cell sequencing. Such is shown through the difference between the sham vs WT model.

When looking at markers derived separately between TBI vs Sham and 5xFAD vs WT, there were only 4 genes in common -- all genes attributed to the regulation of inflammatory response (Figure 2). All four genes belong to a group of markers of highly activated microglia (HAM) that in previous studies has been related to

neuroinflammation in aged mice ((C. Jin, Y. Shao, X. Zhang, J. Xiang, R. Zhang, Z. Sun, S. Mei, J.Zhou, J. Zhang, L. Shi. A Unique Type of Highly-Activated Microglia Evoking Brain Inflammation via Mif/Cd74 Signaling Axis in Aged Mice. *Aging and disease*, **12**, 2125–2139 (2021).)). Further, three out of the four genes are DAM markers ((A. Grubman, X. Y. Choo, G. Chew, J. F. Ouyang, G. Sun, N. P. Croft, F. J. Rossello, R. Simmons, S. Buckberry, D. V. Landin, J. Pfluege, T. H. Vandekolk, Z. Abay, Y. Zhou, X. Liu, J. Chen, M. Larcombe, J. M. Haynes, C. McLean, S. Williams, S.Y. Chai, T. Wilson, R. Lister, C.W. Pouton, A.W. Purcell, O.J.L. Rackham, E. Petretto, J. M. Polo. Transcriptional signature in microglia associated with AB plaque phagocytosis. Nature Communications, **12**, 3015 (2021)
)). This fits with the already established literature on the existence of neuroinflammation in TBI and FAD.

When looking at markers that were not shared between the datasets, we found that, though gene markers are different, the same GO terms are still observed, for example, “neutrophil degranulation”. Also, there exist many terms with similar pathways or functions, for example, “regulation of leukocyte activation” and “myeloid leukocyte activation” (Figure 3). Indeed most of the terms are inflammation related, showing the importance of inflammation in FAD and TBI microglia, and indicating the overall importance of the inflammatory-related process in both the 5xFAD and TBI pathology. This directs to the possibility of controlling inflammation as a potential therapeutic strategy. This may also indicate that drug treatments targeting these genes for neuroinflammation may be translatable across multiple diseases.

Although we have identified inflammation-related gene ontologies and shared genes, these findings require validation. More details will be needed to understand and trace the specific pathways involved in the microglial transcriptional profile of TBI and FAD.

Conclusion and Future Implications

In this study, we compared the microglial processes in TBI and FAD, hoping to find microglial signatures that may extend to neurodegeneration in general. By identifying markers of overlapping microglial populations in these two conditions, we identify potential therapeutic targets for multiple diseases. Further research on this topic will require more controls in order to identify more precise differences in the microglia characteristics. Furthermore, a more detailed look into the pathway of the markers of TBI and FAD is needed to further understand the precise difference between TBI and FAD. For example, a potential direction could be to knock out shared genes and observe phenotypical changes in both models. Another possibility is to introduce microglia from TBI and FAD to the alternative condition and observe phenotypical changes. In conclusion, we have identified the relevance of inflammation in both disease processes while also indicating the difference in microglia signature.

Methods:

Single-cell sequencing data GSE175430 ((K. Somebang, J. Rudolph, I. Imhof, L. Li, E. C. Niemi, J. Shigenaga, H. Tran, T. M. Gill, I. Lo, B. A. Zabel, G. Schmajuk, B. T. Wipke,

S. Gyoneva, L. Jandreski, M. Craft, G. Benedetto, E. D. Plowey, I. Charo, J. Campbell, C. J. Ye, S. S. Panter, M.C. Nakamura, W. Eckalbar, C. L. Hsieh. Ccr2 deficiency alters activation of microglia subsets in Traumatic Brain Injury. *Cell Reports*, **36**, 109727 (2021).)) was used for TBI and GSE140510 ((Y. Zhou, W.M. Song, P.S. Andhey, A.Swain, T. Levy, K. Miller, P. Poliani, M. Cominelli, S. Grover, S. Gilfillan, M. Cella, T. Ulland, K. Zaitsev, A. Miyashita, T. Ikeuchi, M. Sainouchi, A. Kakita, D.A. Bennett, J.A. Schneider, M.R. Nicholas, S.A. Beausoleil, J.D. Ulrich, D.M. Holtzman, M.N. Artyomov, M. Colonna. Human and mouse single-nucleus transcriptomics reveal TREM2-dependent and TREM2-independent cellular responses in Alzheimer's disease. *Nat Med,* **26**,131-142 (2020).)) was used for FAD. Data is processed using Seurat to filter out samples with high mitochondria percentage and low number of expressions ((Y. Hao, S. Hao, E. Andersen-Nissen, W. M. Mauck, S. Zheng, A. Butler, M. J. Lee, A. J. Wilk, C. Darby, M. Zager, P. Hoffman, M. Stoeckius, E. Papalexi, E. P. Mimitou, J. Jain, A. Srivastava, T. Stuart, L. M. Fleming, B. Yeung, A.J. Rogers, J.M. McElrath, C.A. Blish, R. Gottardo, P. Smibert, R. Satija. Integrated Analysis of multimodal single-cell data. *Cell*, **184**, (2021).)). The data in the FAD sample was further processed to leave only leukocytes by looking at gene expression of Csf1r and Trem2. The leukocyte-related clusters TBI and FAD samples were integrated separately to extract markers. TBI and FAD samples were then integrated to minimize the batch effect and to observe the similarities between TBI and FAD samples using scaledata, runPCA, runUMAP, FindNeighbors and FindClustors The dimension reduction is observed using UMAP to visualize clustering and observe similarities between clusters. Relevant markers are found through FindAllMarkers with the test being wilcox, only.pos set to TRUE, min.pct set to 0.1, and logfc.threshold set to 0.25. Gene Ontology analysis was conducted using metascape to understand the function of upregulated genes. Heatmaps were created using the Zscore of average expression ((Y. Zhou, B. Zhou, L. Pache, M. Chang, A.H. Khodabakhshi, O. Tanaseichuk, C. Benner, S.K. Chanda. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun*., **1523,** (2019).)).

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