A comparative study of microglial transcriptomes in Traumatic Brain Injury and Familial Alzheimer's Disease reveals key genes involved in inflammatory response

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Microglia, the resident macrophage in the central nervous system, can evolve into disease-associated microglia (DAM), a specific subtype that is seen across multiple neurodegenerative diseases. To further understand microglial states in neurodegeneration, we compared the transcriptional programs of microglia following traumatic brain injury (TBI) and in Alzheimer's Disease (AD) 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 DAM. Four genes in particular, Cst7, Lyz2, Lpl, and Apoe, are upregulated in both AD and TBI. These genes could serve as potential therapeutic targets to target neuroinflammation in multiple neurodegenerative diseases.

Introduction

Microglia, the resident macrophage in the central nervous system, plays an important role in the immune system and, more specifically, in neuroinflammation[1]. In their healthy state, microglia phagocytose debris. However, prolonged stimulation of microglia might activate microglia, causing them to switch to a proinflammatory phenotype that might be harmful to neurons[2]. Two conditions, traumatic brain injury (TBI) and Alzheimer's Disease (AD), adopt this inflammatory state of microglia[3].

Alzheimer's disease, a neurodegenerative disease with progressive neuronal loss, is prevalent in 1 in 10 Americans over 65 years old and contributes to over 100,000 deaths a year[4]. 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 to sporadic AD[5]. Two features of AD are amyloid beta plaques and neurofibrillary tangles, which are accumulations of the proteins amyloid beta and tau, respectively. Prolonged inflammation caused by these accumulations has been identified as harmful to neurons[3,6]. 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)[3,7]. 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[3].

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, contusions, et cetera[8]. Common effects include lifelong motor, cognitive, and behavioral disabilities resulting from neuron death and are observed directly from trauma and secondary pathways[9]. Though primary injuries, injuries 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[10]. 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[3].

Regarding the relationship between TBI and AD, an acute increase of amyloid beta load has been observed after TBI, and as many as 30% of the patients with TBI develop amyloid beta plaques similar to early-stage AD[11]. Furthermore, Tau pathology was also observed in $1/3$ of TBI patients after one or multiple injuries. Inflammasome activity, inflammatory cytokine release, pyroptosis, chronic microglia activation, and neuronal damage have been observed in TBI and have been thought to contribute to the progression of AD[12]. Chronic inflammation is believed to exacerbate Tau and Amyloid Beta pathology[13].

To further understand the role of inflammation in neurodegeneration and the connection between the two diseases, a

comparison of the characteristics of neuroinflammation in AD and TBI needs to be established. Previous studies have identified Ccr2 as a damaging neuroinflammatory gene in TBI[10], while the ligand of Ccr2, Ccl2 was also previously shown to be highly upregulated in the 5xFAD model. However, there is currently no literature establishing the relationship between TBI and AD in the microglia transcriptional profile. Here, we compare single-nuclei or single-cell sequencing data from TBI and 5xFAD mice and find that both TBI and FAD contain markers of highly activated microglia and, despite overall 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

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[10,14]. Zhou et al. collected nuclei from the brains of 7-month-old male and female 5XFAD and WT mice for sequencing via single-*nucleus* RNA sequencing (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 single-*cell* RNA sequencing (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). We integrated TBI with Sham and FAD with WT from each study to identify specific markers for each condition. We identified 17 clusters for TBI vs Sham (Supplementary Figure 2) and 21 for FAD vs WT (Supplementary Figure 2).

There are a few key differences between the two datasets to acknowledge before we make any conclusions. First, because Zhou et al. performed single-nucleus sequencing, while Hsieh et al. performed single-cell sequencing, some genes might be expressed differently in the cytoplasm and the nucleus, some genes expressed in the Hsieh et al. dataset may not appear in the Zhou et al. dataset. Further, due to the inherent differences in these models, with FAD being a disease of aging, cells in the FAD dataset were collected at 7 months of age, while those in the TBI dataset were collected at 3-4 months. This might present further differences in gene expression induced by normal aging. Lastly, the FAD samples contained both males and females while TBI samples contained males exclusively, leading to possible sex differences from the lack of females in the latter study. Because these technical differences could introduce a lot of non-biological variances, it is difficult to make definitive conclusions regarding the differences between the two datasets. However, any similarities identified between the two datasets in spite of these drastic differences in the models are more compelling.

To understand the similarities of these cell types, we integrated microglia from all the datasets (TBI, Sham, FAD, WT). We observed minimal overlap between the datasets, indicating that the characteristics of the microglia isolated in these datasets are not particularly similar, likely due to the limitations stated above (Figure 1 A). When looking at the average expression of differentially expressed genes across genotypes, we similarly observed few differentially expressed genes overlapping between TBI and FAD (Figure 1 B). To directly explore any 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 C). These results highlight that, while there are few similarities in microglial subtypes identified between the TBI and FAD datasets, these are likely due to technical differences in sample collection.

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

To isolate similarities in the microglial response to these neurodegenerative conditions, we identified markers for TBI and FAD compared to their respective controls and created a Venn diagram of marker genes with $avg\ log2FC$ greater than 0 (Figure 2 A). Using this method, we found 4 overlapping upregulated genes that define TBI and FAD microglia ($Cst7$, adjusted $p-value < 2.22\times10^{-308}$; $Lyz2$, adjusted $p-value < 2.22\times10^{-308}$; Lpl, adjusted $p-value = 7.33\times10^{-170}$; Apoe, adjusted $p-value = 1.16e-\times10^{-185}$) (Figure 2A, B). Lpl correlates with microglia reactivity while Cst7, Lyz2, and Apoe are disease-associated microglia (DAM) genes observed in multiple neurodegenerative diseases[15,16]. More interestingly, Lpl and Cst7 are exclusively expressed in highly activated microglia, a subtype that triggers 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[17]. The functionality of these genes was mapped using gene ontology (GO) and indicated that they regulate inflammatory responses (Figure 2 C), consistent with previous literature[10]. These data show that, while the TBI and FAD datasets do not overlap significantly, there is an overlap in the transcriptional states of the microglia.

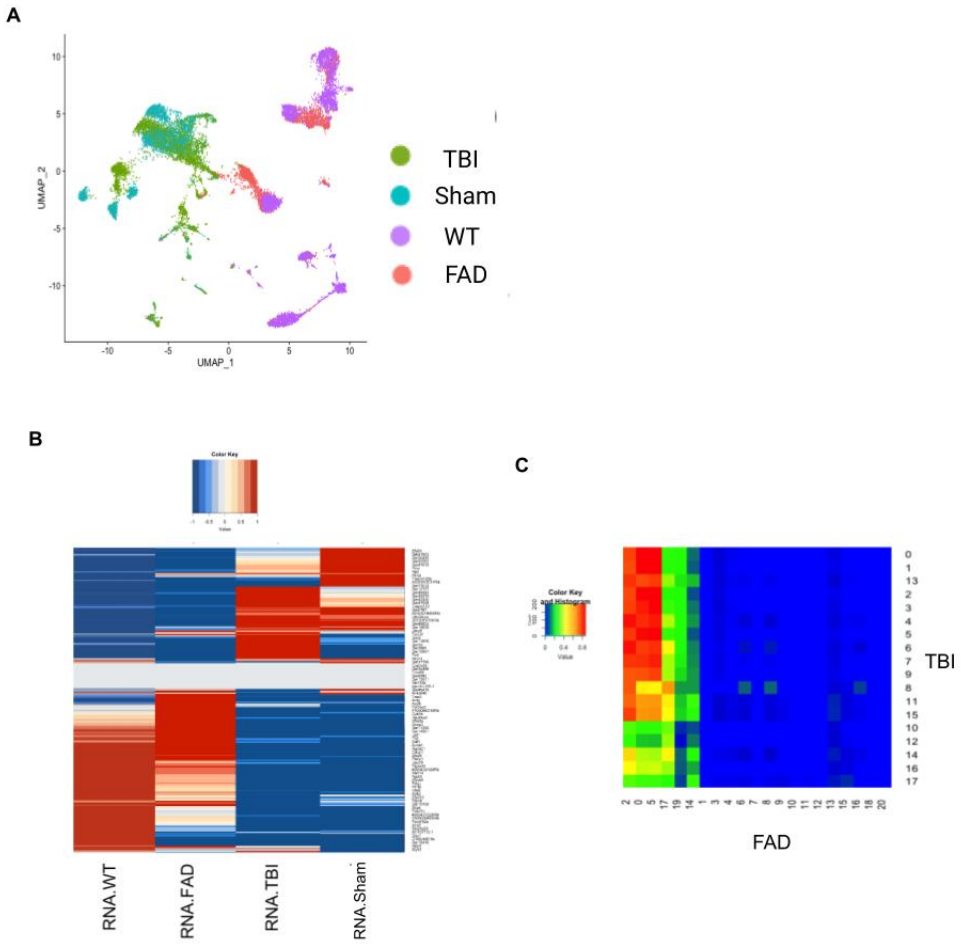

Fig. 1 FAD and TBI microglia do not transcriptionally overlap (A) UMAP of FAD and TBI microglia integrated. (B) Heatmap showing top markers for each disease condition and their respective controls. (C) Correlation plot between FAD (column) and TBI (row) clusters.

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

We further looked into the functionality of upregulated genes that differ between TBI and FAD in the leukocyte population by performing GO analyses on genes not shared by the two datasets (Figure 2A). 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 preserved a similar identity on a broader scale (Figure 3). The majority of the terms are focused on cytokine and inflammation. This reveals that, despite the major differences observed in the UMAPs, the functionality of the upregulated genes is similar. However, the precise reason for such discrepancy needs to be further investigated.

Discussion

To understand the role of microglia in neurodegenerative conditions, we compared a published single-cell RNA sequencing dataset from the 5xFAD mouse model of Alzheimer's disease to that of a controlled cortical impact model of TBI and their respective controls. We find that the microglia signature is relatively different between TBI and FAD as shown through the UMAP (Figure 1), though this is likely due to 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 have further contributed to the difference: 5xFAD is sequenced through single-nuclei sequencing while the TBI model is sequenced

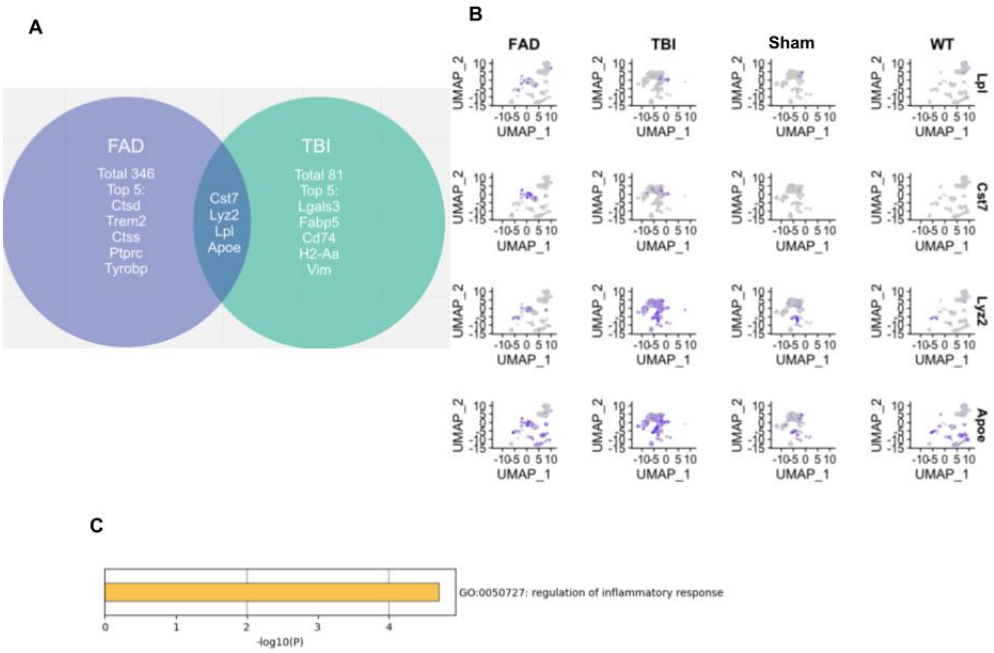

Fig. 2 Four shared markers of microglia in TBI and FAD show the importance of regulation of inflammatory response in both diseases. (A) Upregulated genes were selected with the criteria of $avg\ log2FC > 0$ for both TBI vs Sham and FAD vs WT. (B) Feature plots of the four overlapping genes shown by genotype. (C) GO analysis of overlapping genes reveals a single significant GO term.

through single-cell sequencing. These technical differences themselves lead to differential gene expression between these two studies, as evidenced by the comparison of WT and sham in Figure 1B. This means that the result obtained from the UMAP is potentially not representative of the similarities and differences between the two diseases, however, it further highlights that any results obtained from similarities would have more significance.

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[17]. Further, three out of the four genes are DAM markers[15]. This fits with the already established literature on neuroinflammation in TBI and FAD. It is then likely that these 4 genes directly influence or play a major role in the signaling pathway producing inflammasomes like NLRP1, NLRP3, AIM2, and ASC speck activity. To further understand the roles of these four genes in these neurodegenerative conditions, microglial knockouts of these genes in mouse models can be informative.

When looking at markers that were not shared between the datasets, we found that, though the majority of the 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 related to inflammation, highlighting the importance of inflammation in microglia, and indicating the overall importance of the inflammatory process in FAD 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.

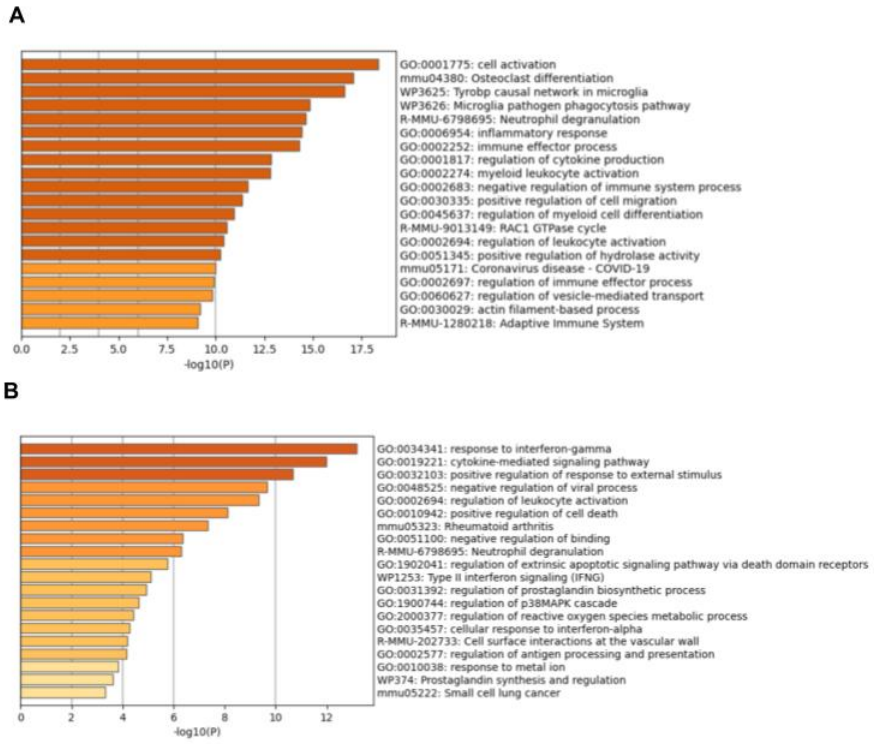

Fig. 3 GO terms of unique upregulated genes in TBI and FAD shows various inflammation-related terms in both diseases despite the distinct microglia expression profiles. GO terms of upregulated genes in FAD (A) and TBI (B) that are not shared between genotypes.

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. 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 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 indicating the difference in microglia signature.

Methods

Data pre-processing

Single-cell sequencing data GSE175430[10] was used for TBI, and GSE140510[14] was used for FAD. Data is processed using Seurat to filter out samples with high mitochondria percentages and a low number of expressions[18]. The data in the FAD sample was further processed to leave only leukocytes by looking at the gene expression of *Csf*1*r* and *Trem*2. The leukocyte-related clusters TBI and FAD samples were integrated separately to extract markers.

Data integration and differential expression analysis

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 Wilcoxon Rank Sum test, only.pos set to TRUE, min.pct set to 0.1, and logfc.threshold set to 0.25. Heatmaps were created using the Z-score of average expression across cells.

Correlation heatmap

We identified the 500 most highly variable features in each dataset (FAD and TBI) to create a correlation heatmap. The function cor (CRAN) was used to compute a correlation score for each cluster, and each score was plotted in a heatmap from blue to red.

Gene Ontology analysis

Gene Ontology analysis was conducted using Metascape[19] (http://metascape.org) to understand the function of upregulated genes.

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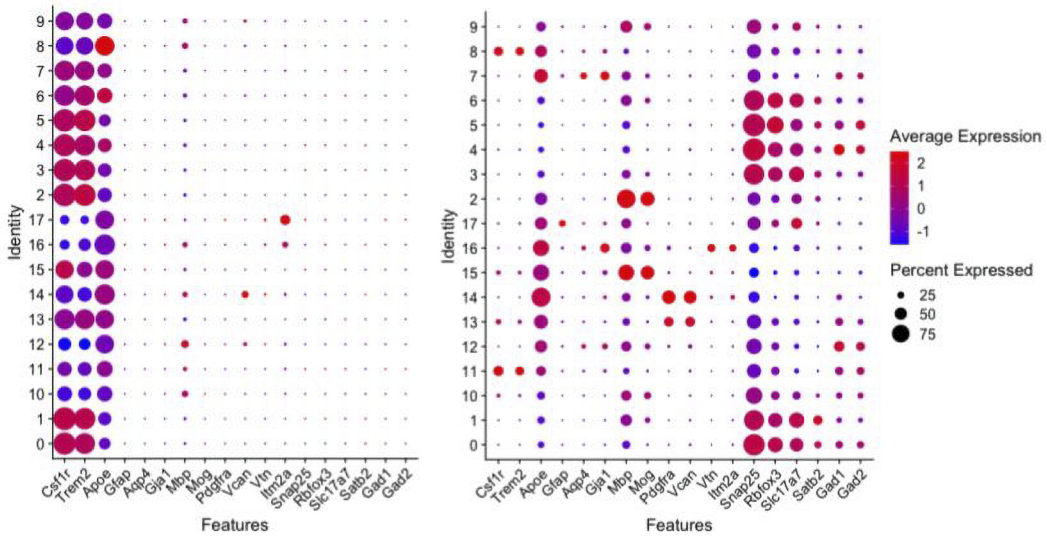

Fig. S1 Dot plot of TBI and FAD datasets (Csf1r, Trem2, Apoe, Gfap, Aqp4, Gja, Mbp, Mog, Pdgfra, Vcan, Vtn, Itm2a, Snap25, Rbfox3, Slc17a7, Satb2, Gad1, Gad2).

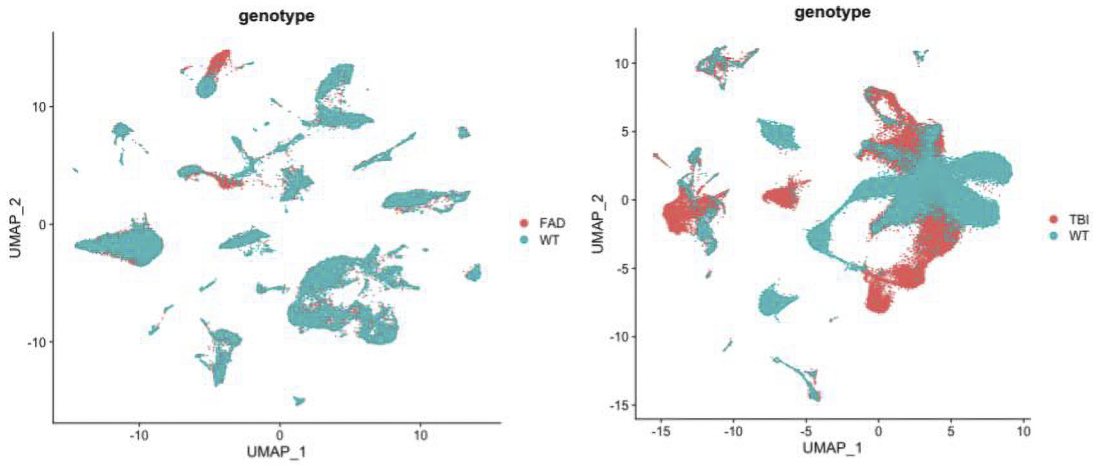

Fig. S2 UMAPs of microglia subsetted by expression levels of Csf1r and Trem2 from the FAD and TBI datasets.