

T.R
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL

**CELL SUBTYPE SPECIFIC ROLES OF GLIAL CELLS IN
ALZHEIMER'S DISEASE BY NETWORK-BASED ANALYSIS OF
SINGLE-CELL MULTI-OMIC DATA**

ATILAY İLGÜN

A THESIS OF MASTER OF SCIENCE
DEPARTMENT OF BIOENGINEERING
BIOINFORMATICS AND SYSTEMS BIOLOGY PROGRAM

ADVISOR: PROF. DR. TUNAHAN ÇAKIR

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LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

**TEK HÜCRELİ ÇOKLU OMİK VERİLERİN AĞ
TABANLI ANALİZİ İLE ALZHEIMER HASTALIĞINDA
GLİAL HÜCRELERİN HÜCRE ALT TİPİNE ÖZGÜ
ROLLERİ**

ATILAY İLGÜN

YÜKSEK LİSANS TEZİ
BİYOMÜHENDİSLİK ANABİLİM DALI
BİYOİNFORMATİK VE SİSTEM BİYOLOJİSİ
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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder, and it is the most common cause of dementia. Many different factors contribute to the onset and pathology of the disease. Recent studies have revealed incontrovertible roles of astrocytes in the pathology of AD. Considering the conflicting behaviours of astrocytes in AD brain, they have been proposed to have subtypes. Comprehensive single-cell based datasets have been generated to characterize cell subtypes for different tissues. In this study, two publicly available single-nuclei transcriptome datasets based on post-mortem human brain prefrontal cortex from AD patients and healthy controls were re-analysed. Subsequently, astrocytes from both datasets were integrated to provide in depth characterization of astrocyte subtypes in AD. Differentially expressed genes within each astrocyte subtype were analysed by mapping them onto a human protein-protein-interaction network to discover subnetworks with biologically relevant genes. The single-nuclei data integration and network-based analysis methods implemented in this study showed higher sensitivity in capturing disease related genes compared to traditional approaches. The genes in the discovered subnetworks were subjected to functional enrichment analyses, and subtype-specific altered biological processes were identified in AD. It was found that subtype-2 AD astrocytes exhibited neuroprotective behaviour while subtype-3 AD astrocytes represented neurotoxic reactive astrocytes. A1 reactive astrocytes were shown to have an enhancing role for the neurofibrillary tangle accumulation through MAPK10, MAPT and TMED10, which were uniquely upregulated in subtype-3 during AD. Moreover, snATAC-seq data for the same tissue was re-analysed and astrocytic cells were identified to evaluate astrocyte subtypes at multi-omic level. It was found that astrocyte subtypes underwent epigenetic reprogramming during AD. Potential transcription factors were also identified for the regulation of the genes that exhibited alterations in both promoter accessibility and gene expression in AD. It was found that PTN gene, which was reported to be important for AD pathology, might be regulated by ATF3 transcription factor in subtype-1.

Keywords: Single-nuclei, Transcriptome, Epigenome, Alzheimer's Disease, Astrocytes

ÖZET

Alzheimer hastalığı (AH) neurodejeneratif bir rahatsızlıktır ve demansın en yaygın sebebidir. Hastalığın oluşmasına ve patolojisine katkı yapan bir çok faktör bulunmaktadır. Son yapılan çalışmalar astrositlerin AH'ye katkılarının yadsınamaz olduğunu ortaya koymuştur. Astrositlerin AH'li beyindeki raporlanan farklı davranışları değerlendirildiğinde, alt tiplerinin olduğu tahmin edilmektedir. Farklı dokulardaki hücre alt-tiplerini karakterize edebilmek amacıyla kapsamlı tek-hücre dizileme verisetleri oluşturulmaktadır. Bu çalışmada, postmortem AH'li hastaların ve sağlıklı kontrollerin beyin prefrontal korteksinden alınan örneklerle elde edilen iki farklı tek-çekirdek transkriptom veriseti yeniden analiz edildi. Bu doğrultuda, AH'deki astrosit alt tiplerinin derinlemesine karakterizasyonunu sağlamak için her iki veri setinde tespit edilen astrositler birleştirildi. Her astrosit alt tipi için anlamlı ifade edilen genler, insan protein-protein-etkileşim ağına haritalandı ve biyolojik olarak ilişkili genlerden oluşan öbeklerin keşfi gerçekleştirildi. Bu çalışmada uygulanan tek çekirdekli veri entegrasyonu ve ağ tabanlı analiz yöntemleri, geleneksel yaklaşımlara kıyasla hastalıkla ilişkili genlerin yakalanmasında daha yüksek hassasiyet göstermiştir. Öbek genleri, gen ontoloji analizlerine tabi tutuldu ve AH'de her alt-tipe özgü değişen biyolojik yollar belirlendi. Altıtip-2'de yer alan AH astrositlerinin nöroprotektif davranış sergilediği, altıtip-3 AH astrositlerinin ise nörotoksik reaktif astrositleri temsil ettiği bulundu. A1 reaktif astrositlerin, AH sırasında altıtip-3'e özel artış gösteren MAPK10, MAPT ve TMED10 yoluyla nörofibriler yumak birikimini nasıl artırabileceği de değerlendirildi. Ayrıca aynı dokuya ait tek çekirdekli ATAC-seq verilerin de yeniden analizi gerçekleştirildi ve astrosit alt tipleri bu veriler için de tanımlanarak çoklu omik düzeyinde karşılaştırmalar yapıldı. Astrosit alt tiplerinin AH sırasında epigenetik yeniden programlanmaya maruz kaldığı gösterildi. Ayrıca AH'de hem promotör erişilebilirliğinde hem de gen ifadesinde değişiklikler sergileyen genleri düzenleyebilecek potansiyel transkripsiyon faktörleri de belirlendi. AH patolojisi açısından önemli olduğu bildirilen PTN geninin altıtip-1'de ATF3 transkripsiyon faktörü tarafından düzenlenebileceği gösterildi.

Anahtar kelimeler: Tek-çekirdek, Transkriptom, Epigenom, Alzheimer Hastalığı, Astrositler

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LIST OF SYMBOLS AND ABBREVIATIONS

AD	: Alzheimer's Disease
APP	: Amyloid Precursor Protein
ATAC-seq	: Assay for Transposase Accessible Chromatin-sequencing
A β	: Amyloid Beta
BBB	: Blood Brain Barrier
CCA	: Canonical Correlation Analysis
CFAS	: Cognitive Function and Ageing Study
CNS	: Central Nervous System
DAM	: Disease Associated Microglia
DEG	: Differentially Expressed Genes
DGEA	: Differentially Gene Expression Analysis
GEO	: Gene Expression Omnibus
KPM	: KeyPathwayMiner
LSI	: Latent Semantic Indexing
ND	: Neurodegenerative Disorder
NFTs	: Neurofibrillary Tangles
PCA	: Principle Component Analysis
PCs	: Principle Components
PPI	: Protein-Protein Interaction
RNA-seq	: RNA-sequencing
ROSMAP	: Religious Orders Study and Rush Memory and Aging Project
SCE	: Single Cell Experiment
snATAC-seq	: Single nuclei ATAC-seq
SNN	: Sharest Nearest Neighbors
snRNA-seq	: Single-nuclei RNA-seq
tSNE	: t-Distributed Stochastic Neighbor Embedding
ZINB-WaVE	: Zero-Inflated Negative Binomial-based Wanted Variation Extraction

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1. INTRODUCTION

Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder (ND) that is typically characterized by massive amount of neuronal loss in the brain, and it is considered to be a global health concern. According to World Health Organization, more than 55 million people have dementia worldwide, with the most common cause of being AD. It affects millions of lives directly and indirectly, and knocks back economies of countries worldwide revealing the devastating socio-economic sides of the disease. AD destroys thinking and memory skills and leads to cognitive impairment. Patients who suffer from AD, particularly in late stages, might become bedridden accompanied by swallowing and aspiration problems. Type 2 diabetes, obesity, traumatic brain injuries, stress, age, infections, smoking, air pollution, and genetic factors are potential risk factors for AD (X. X. Zhang et al., 2021). AD is characterized by abnormal accumulation of amyloid-beta ($A\beta$) and tau proteins as well as synaptic loss (Serrano-Pozo et al., 2011) and neuronal degeneration.

Although AD is considered to be a neuron-based disorder, the fact that risk factor genes are mainly expressed by glial cells reveals their undeniable roles on AD pathology. Particularly, astrocytes, most abundant type of glial cells in central nervous system (CNS), were proven to have strong modulatory impacts on AD pathology. Studies focusing on astrocytes in AD revealed that their responses might be either neuroprotective or neurotoxic (Preman et al., 2021). A possible explanation for their heterogenous roles in AD is that astrocytes might indeed have subtypes. For a proper characterization of astrocyte subtypes, comprehensive single-cell genomics approaches and high amount of astrocytes were required because small populations cannot adequately reflect the transcriptional dynamics during AD. Although astrocytes are one of the most abundant cell types in CNS, low capturing rate of astrocytes causes insufficient reflection of disease conditions. For this reason, Sadick et al. 2022 developed an astrocyte enrichment strategy to increase the number of captured astrocytes up to 10-fold per donor and conducted single-nuclei transcriptomic profiling of post-mortem human brain prefrontal cortex astrocytes (Sadick et al., 2022). They profiled more than 40,000 astrocytic nuclei from healthy and AD conditions and identified nine astrocyte subtypes with unique transcriptional signatures.

In this study, publicly available single-nuclei transcriptome datasets (Morabito et al., 2021; Sadick et al., 2022) based on post-mortem human brain prefrontal cortex from AD patients and healthy controls were re-analyzed, and astrocytes from both datasets were integrated to provide in depth characterization of astrocyte subtypes in AD. Then, differentially expressed genes between control and AD astrocytes within each subtype were mapped onto a human protein-protein interaction network to generate subnetworks with biologically relevant genes. The findings were reinforced by analyzing snATAC-seq based astrocyte data available for the same tissue in one of the studies (Morabito et al., 2021), enabling the identification of chromatin accessibility information for the astrocyte subtypes as well as differentially accessible regions.



2. BACKGROUND INFORMATION

2.1. Alzheimer's Disease

AD is a polygenic neurodegenerative disorder named after Alois Alzheimer. AD destroys thinking and memory skills of the patients and leads to cognitive impairment. Both genetic and environmental factors contribute to the onset and pathology of the disease. Abnormal accumulation of A β and hyperphosphorylated tau proteins are the molecular hallmarks of the disease. Their accumulation leads to the generation of amyloid plaques and neurofibrillary tangles (NFTs), respectively. While amyloid plaques occur in intercellular space, NFTs occur inside neurons. A β peptides are generated by the cleavage of amyloid precursor protein (APP). In fact, APP protein can be cleaved through different enzymes such as α -, β - and γ - secretases. Different paired combinations of these enzymes activate either amyloidogenic or non-amyloidogenic pathways. While non-amyloidogenic pathway (α - and γ - secretases) does not lead to the formation of A β , amyloidogenic pathway (β - and γ - secretases) can lead to the formation of A β and, subsequently, amyloid plaques. In AD, amyloidogenic pathway involves and amyloid plaques are generated. Deposition of A β and hyperphosphorylated tau disrupts cellular homeostasis in various ways, causing neuroinflammation and neurodegeneration. Despite the fact that many different approaches have been developed to slow down the progression of the disease (cholinesterase inhibitors, monoclonal antibody-based approaches that target amyloid plaques etc.), there is no definitive therapy for AD.

AD was considered to be a neuron-based disorder, but recent studies revealed the regulatory roles of glial cells in AD pathology. Development of the single-cell sequencing platforms enabled analysis of cell-specific regulations of glial cells during AD. Particularly, astrocytes, due to their wide variety of functions, have important contributions to AD pathology and are highly affected by the disease. To enlighten the onset and progression of the disease, systematic contributions from each cell type must be deeply investigated through systems biology related approaches.

2.2. Astrocyte Development

Astrocytes are originated from radial glia, which were derived from neuroepithelium (Preman et al., 2021). In prenatal stages (before birth), radial glial cells undergo asymmetric division to generate astroglial progenitors, which subsequently generate astrocytes (Preman et al., 2021). However, a major proportion of astrocytes is generated postnatally by the symmetric division of differentiated astrocytes (Preman et al., 2021). Figure 2.1 shows the development of astrocytes from embryonic stage to adult stage. Three major signalling pathways regulate embryonic astrocyte development: JAK/STAT, Notch and BMP-SMAD. For the initiation of astrogenesis, CNTF, CT-1 and LIF cytokines are necessary as they activate JAK/STAT pathway (Nakashima et al., 1999) and activated STAT promotes the transcription of astrocyte-related genes (F. He et al., 2005). In the beginning of the astrogenesis, Notch signalling pathway also regulates GFAP epigenetically, and allows its transcription (Preman et al., 2021). Moreover, JAK/STAT and Notch signalling pathways can be activated by BPM signalling (Nakashima et al., 1999; Takizawa et al., 2003).

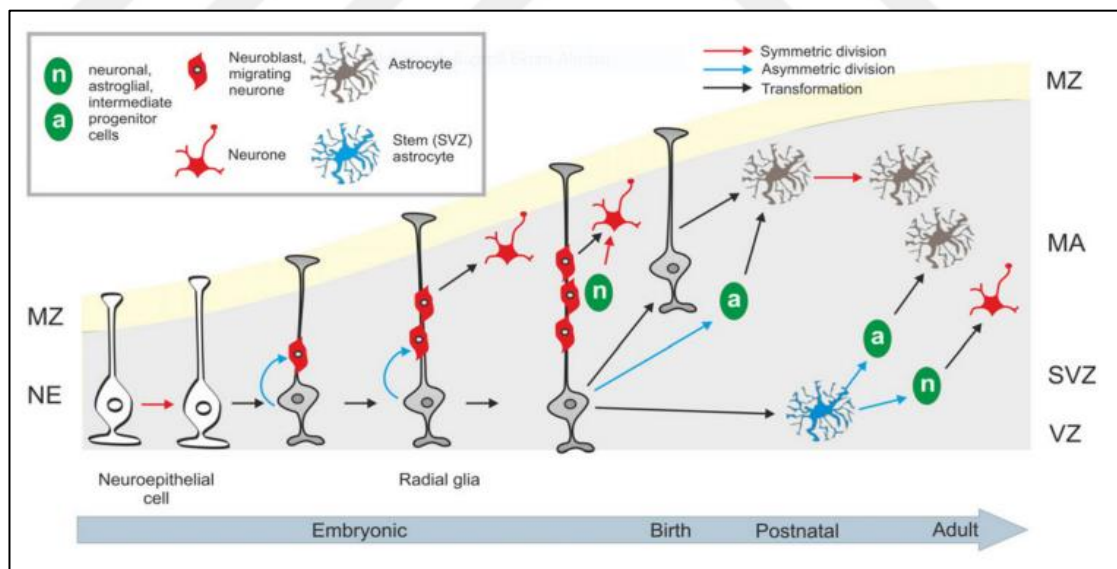


Figure 2.1: Astrocyte development from embryonic phase to adult phase (Preman et al., 2021).

As a summary, astrocytes are generated in different developmental stages like pre- and postnatal stages. Radial glia, glial progenitors and OPCs can generate astrocytes.

2.3. Astrocytes in Central Nervous System

Astrocytes perform variety of functions in brain; regulating ion homeostasis, neurotransmitter clearance (GABA, glutamate) and prevent excitotoxicity (Vasile et al., 2017). They also regulate synapse formation, synaptic transmission and synapse maintenance. They are also able to eliminate synapses by phagocytosis (Lee et al., 2021). They are also involved in the structure of Blood-brain barrier (BBB) by their end-feet together with endothelial cells and pericytes. Astrocytes also provide trophic factors and energy to neurons. Astrocytes also support neurons by metabolically (Figure 2.2) (Newington et al., 2013).

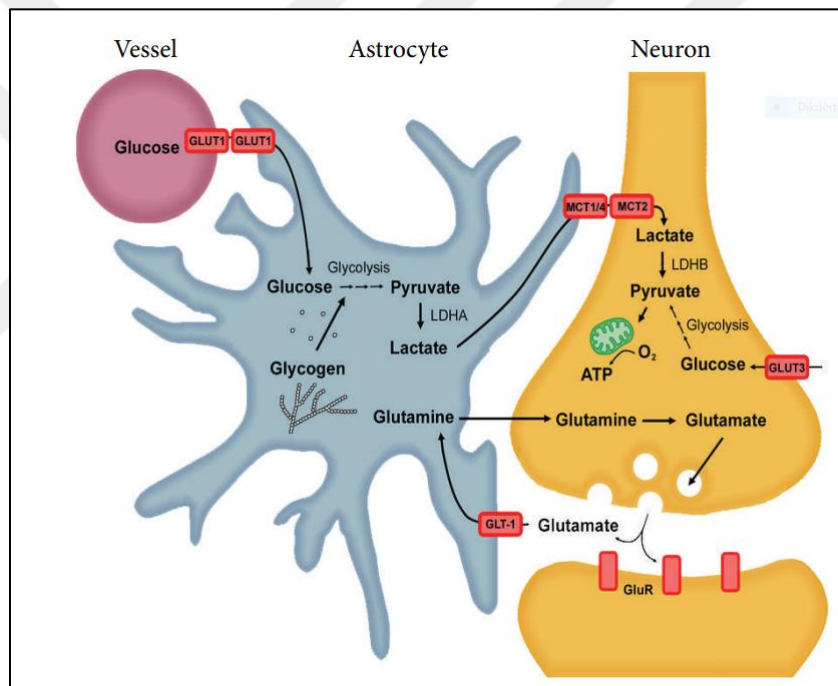


Figure 2.2: Astrocytes metabolically support neurons. (Newington et al., 2013)

Glycogen stored by astrocytes can be metabolised into pyruvate and lactate, and it can be transferred into surrounding neurons (Preman et al., 2021). Moreover, glutamate neurotransmitter secreted by neurons, taken up by astrocytes and converted into glutamine, and glutamine can be transferred back to neurons. By this way neurotransmitter recycling is held by astrocytes. Astrocytes are also closely associated with blood vessels there they take certain metabolites like iron which is used as a cofactor for many reactions in myelin production.

2.4. Astrocytes in Alzheimer's Disease

As it was described above, astrocytes have a wide variety of functions in CNS. Apart from these duties, astrocytes were shown to be involved in AD pathology. Astrocytes were found to be closely associated with amyloid plaques in both cortex of APP/PS1 mice brain and entorhinal cortex of AD patient brain (Figure 2.3) (Preman et al., 2021).

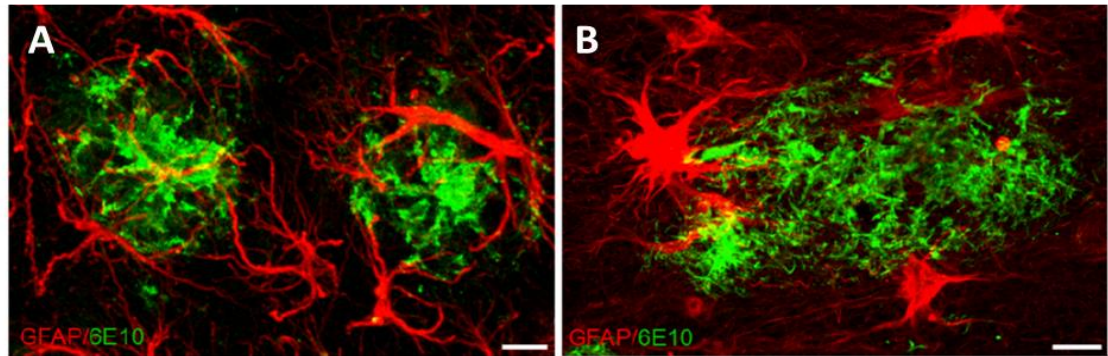


Figure 2.3: Association of astrocytes with amyloid plaques. GFAP-positive mouse (A) and human astrocytes (B) were shown in red, and amyloid plaques were given in green color (Preman et al., 2021).

Abnormal accumulation of A β in intercellular space has been shown to alter many different biological processes in astrocytes, including neurotransmitter clearance, calcium signaling and gliotransmission (González-Reyes et al., 2017). These altered biological processes might worsen the disease pathology, but astrocytes also have a variety of mechanisms to clear and eliminate A β . For example, they express AQP4 (Aquaporin 4) water channels in their end-feet and by this way they are involved in amyloid beta clearance (Iliff et al., 2012). Moreover, A β clearance is also mediated by APOE, CLU, ACT and α 2-macroglobulin (α 2-M) proteins (Preman et al., 2021). Astrocytes also express proteases (ECE1, ECE2, NEP, IDE) that can degrade A β peptide into smaller fragments (Lin et al., 2018).

Despite the fact that astrocytes modulate events related to A β , thereby assuming a neuroprotective role, in theory, due to the astrocytic upregulation of Amyloid precursor protein (APP) and β -secretase-1 during AD, they can contribute to A β production (Frost & Li, 2017). Moreover, neuroinflammation can promote the production of astrocytic amyloid beta (Frost & Li, 2017). Also, it was shown that A β is able to

activate NF- κ B (Nuclear factor kappa light chain enhancer of activated B cells) signaling pathway in astrocytes, which eventually changes phagocytic properties of microglia and disrupts neuronal morphology (Lian & Zheng, 2016a). When NF- κ B pathway is active, C3 protein is upregulated in astrocytes. Subsequently, C3 is released from astrocytes, and it can bind to its receptor C3a in neurons or microglia and changes phagocytosis and disrupts neuronal morphology respectively (Lian & Zheng, 2016a). As a summary, astrocytes in AD can be both neuroprotective and neurotoxic.

2.5. Development of Single-cell Sequencing Platforms and Their Contribution to AD

Next generation sequencing technology revolutionized the field of molecular biology and led to the emergence of new omic technologies. The development of RNA-seq technology, and implementation of this high-throughput technology at single-cell level, called single-cell RNA-seq, has been proven to contribute to enlighten disease etiologies including AD. Indeed, the number of single-cell-based datasets has been increasing day by day.

The first single-cell study was published in 2009 (Tang et al., 2009), and after this pioneering study, more single-cell sequencing platforms were developed and published (Figure 2.4) (Ahmadi et al., 2021). Single-cell sequencing platforms can be categorized into two distinct groups: droplet-based and plate-based methods. While 10x Chromium Genomics, Drop-Seq, inDrop are droplet-based methods; Smart-Seq, Smart-Seq2, STRT-Seq and MARS-Seq are plate-based methods. In addition to single-cell sequencing, single-nuclei RNA-seq has also emerged in recent years. Although, single-nuclei RNA-seq and single-cell RNA-seq platforms share common points, ability to work with frozen tissue is a major advantage for snRNA-seq platforms.

By the development of single-cell platforms, single-cell based databases have also emerged in recent years, providing access to in-depth characterization of single-cells from various tissues, organs and organisms. By the usage of these databases, cell-type-specific gene expressions can be re-analyzed. Moreover, there are computational tools developed that use the power of single-cell sequencing technologies and integrate information from single-cell data with bulk-transcriptome data, called deconvolution. Deconvolution algorithms allow the prediction of cell-type-specific expression from bulk transcriptome data by using single-cell count matrices.

Single-cell sequencing studies have paved the way for enlightening the etiology of many neurodegenerative diseases, including AD. Lots of stunning results were obtained through the implementation of single-cell sequencing for AD. To illustrate, in 2017 (Keren-Shaul et al., 2017), disease associated microglia (DAM), a subset of microglia that have been proposed to have protective roles, were identified in AD mouse models. There are also many human single-cell based studies that has recently become available (Grubman et al., 2019; Mathys et al., 2019; Morabito et al., 2021; Sadick et al., 2022). One of the datasets (Sadick et al., 2022), also used in this thesis, focused onto astrocyte and oligodendrocyte subtypes in prefrontal cortex during AD, and they found that these cell types undergo subtype-specific transcriptional changes in AD. They profiled 80,247 single-nuclei and 41,071 of them were belong to astrocytes. They also reported gain and loss of functional changes in AD through functional enrichment analyses in both astrocytes and oligodendrocytes. Most importantly, they reported that data integration enhances detection of unique astrocyte subpopulations. Therefore, integration of astrocytes from different datasets enables proper detection of subtypes. In the dataset by Morabito et al. (2021), the other dataset used in this thesis study, they performed profiling of approximately 200,000 single-nuclei through multi-omic approach where 4,756 and 15,339 astrocytes were obtained from snRNA-seq and snATAC-seq respectively. They identified cell-type specific and disease associated cis-regulatory elements. Since snATAC-seq data was also available in this dataset, it allows for implementation of the multi-modal approaches. Therefore, by the integration of Sadick and Morabito datasets, more sensitive subtype detection and identification of epigenetic profiles of subtypes can be conducted in a row. As a summary, single-cell based sequencing platforms have important contributions to enlighten the pathology of AD and by the integration of different datasets and modalities, disease etiologies might be enlightened.

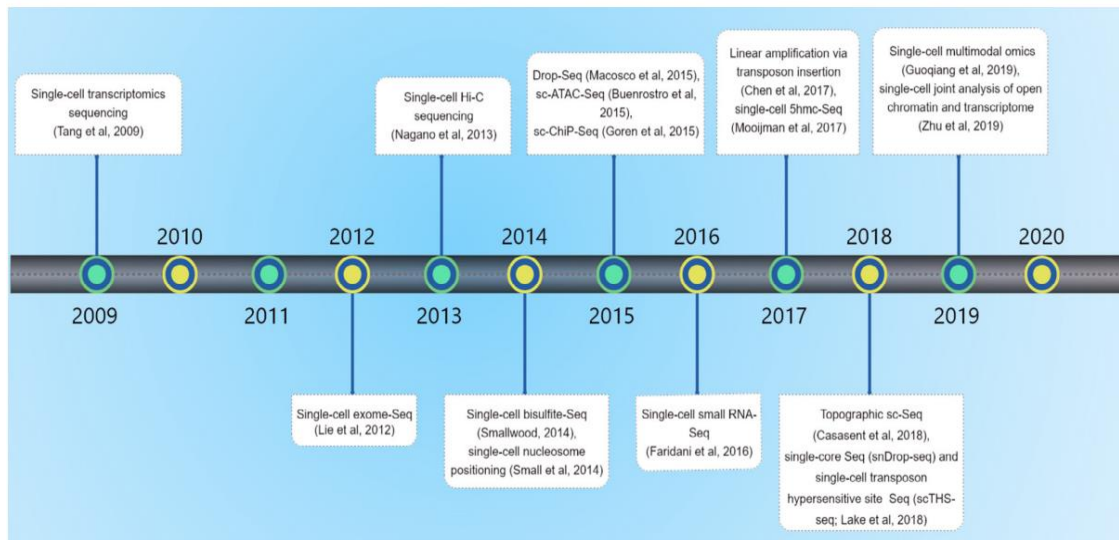


Figure 2.4: Chronological development of single-cell sequencing technologies (Ahmadi et al., 2021).

2.6. KeyPathwayMiner

KeyPathwayMiner (KPM) (List et al., 2016) is a tool for subnetwork discovery from a given organism-specific interaction network with the help of omic data. By using KPM, gene expression data can be mapped onto PPI network of the organism of interest. Main purpose of KPM is to generate subnetworks with biologically relevant genes, in our case AD genes. Also, complex natures of the neurodegenerative diseases can be enlightened. KPM was used in several studies to generate disease-specific subnetworks. For example, In a study, APP protein was identified as the most linked hub gene in Crohns's Disease specific subnetwork (Maden & Acuner, 2021). For this reason, it has been proposed that similar genetic factors might contribute to both AD and Crohn's Disease (Maden & Acuner, 2021). Also, in 2021, Emanetci and Cakir mapped learning and memmory related transcriptome data on PPI network by different subnetwork discovery tools including KPM and discovered learning and cognition-based genes (Emanetci & Çakır, 2021). By their network-based study, they identified genes that were associated with memory that could not be obtained from the transcriptome data (Emanetci & Çakır, 2021). For transcriptome data, the required inputs for KPM are a list of differentially expressed genes and interactome data from the organism of interest. After performing the DEG analysis based on a specified p-value threshold, differentially up and downregulated genes are identified. Typically,

KPM requires all the genes as an input, with the differential expression of these genes indicated in binarized format in a second column, where 0 and 1 represents non-significant and differentially expressed genes respectively. Number of non-significant genes to be allowed in subnetworks were controlled by K parameter. The other input of KPM is interactome data, which can be downloaded from BioGrid database for several organisms. Interactome data can be filtered based on specific criteria, to illustrate, contain only the physically interacting proteins, removal of self loops etc.



3. MATERIALS AND METHODS

3.1. snRNA-seq and snATAC-seq Data Analysis Overview

In this study, two independent snRNA-seq datasets (GSE167494 and GSE174367) (Morabito et al., 2021; Sadick et al., 2022) collected from post-mortem human brain prefrontal cortex tissue of patients with AD and healthy controls were downloaded from the Gene Expression Omnibus (GEO) database. We will refer to these datasets by the names of the first authors in their respective data articles: Sadick and Morabito. While both contain snRNA-seq data, the Morabito study also have snATAC-seq data available for the same tissue. Count and peak matrices of the datasets were imported in R (v4.2.1). The single-nuclei RNA-seq datasets were processed by the comprehensive single-cell genomics tool Seurat (v4.3.0) (Hao et al., 2021). On the other hand, the single-nuclei ATAC-seq dataset was processed by using Signac (v1.7.0) (Stuart et al., 2021) single-cell chromatin data analysis tool. Single-nuclei RNA-seq datasets were analysed independently. After the annotation of major cell types in each dataset, astrocytes were subsetted, integrated and reclustered to determine the subtypes. Meanwhile, snATAC-seq data was processed, annotated, subsetted and reclustered. Then, Seurat's label transfer approach was used to transfer subtype labels in RNA-seq astrocytes to ATAC-seq astrocytes. This enabled chromatin accessibility information for the astrocyte subtypes. Since all three datasets (2 snRNA-seq and 1 snATAC-seq) were processed independently, analysis steps of each of them are explained independently in the following sections in detail, and Figure 3.1 is the graphical representation of the whole methodology.

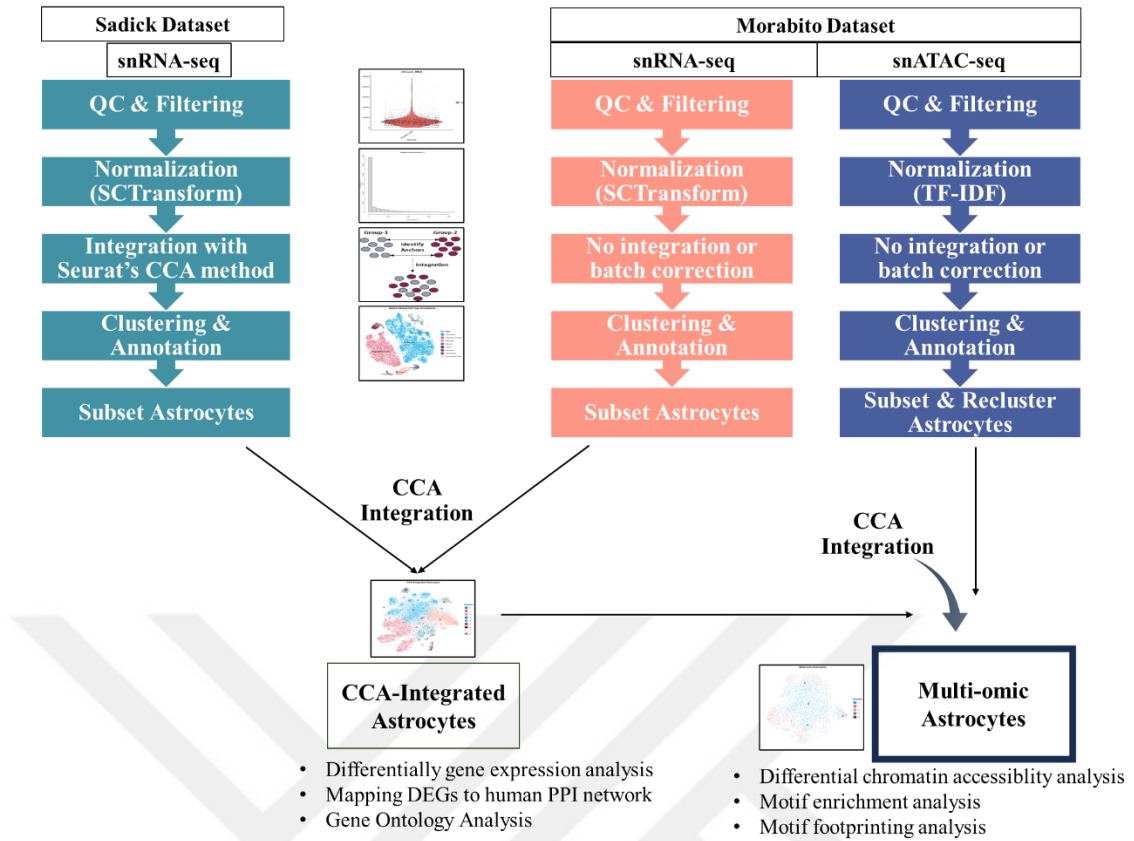


Figure 3.1: Overview of single-nuclei data analysis approach and overall methodology implemented in this study. Each dataset was analysed independently, and after the annotation of CNS cells, astrocytes were integrated. The integrated data was used to identify differentially expressed genes between control and AD groups, followed by their mapping onto the human PPI network. In parallel, snATAC-seq dataset was also analysed and astrocytes were subsetting and reclustering. Subsequently, multi-modal integration was implemented, and differentially accessible regions were identified. This was followed by motif enrichment and foot printing analyses.

3.2. snRNA-seq Data Processing for the Sadick Dataset

Gene expression count matrices from the donors in the Sadick dataset (Sadick et al., 2022) (5 control, 9 AD) were introduced into R independently. Cells in each donor were filtered based on the number of unique transcripts per cell, the total number of transcripts per cell and percentage of mitochondrial genes per cell as recommended by Seurat (see Appendix A.1-5 for the distribution of unfiltered & filtered quality control metrics for the Sadick dataset). After filtering, the raw count matrix of each donor was independently normalized with `sctransform::SCTransform` (v0.3.5) (Hafemeister &

Satija, 2019) function. This function does not only normalize the count data, but it also scales the data and identifies variable features. For each donor, 2000 variable genes were identified, and the percentages of transcripts corresponding to mitochondrial, ribosomal, and heat-shock proteins as well as hemoglobin transcripts were regressed out during this normalization step. Afterwards, `Seurat::SelectIntegrationFeatures` function was used to select a new set of 2000 features from the donor-derived variable features, to be used in integration. Then, transcripts for mitochondrial, ribosomal, and heat-shock proteins and hemoglobin were removed among those variable features, leaving 1989 variable features.

In the next step, the normalized data was prepared for Canonical Correlation Analysis (CCA) (Stuart et al., 2019), the analysis of choice for integrating data from different donors, by `Seurat::PrepSCTIntegration` function. Integration by CCA was performed by using `Seurat::FindIntegrationAnchors` and `Seurat::IntegrateData` functions respectively (dims=50). `Seurat::FindIntegrationAnchors` function was used to identify anchor cells across different donors. Those anchor cells are pair of cells from each donor, and they were used as input for `Seurat::IntegrateData` function. After the integration, Principle Component Analysis (PCA) was implemented for linear dimension reduction by using `Seurat::RunPCA` function, and it was followed by non-linear dimension reduction method t-Distributed Stochastic Neighbor Embedding (tSNE) by using first 50 principle components (PC). Non-linear dimension reduction was implemented by `Seurat::RunTSNE` function and followed by the computation of Shared Nearest-Neighbor (SNN) by using `Seurat::FindNeighbors` function (dims=50).

Subsequently, cell clustering was performed by the Louvain algorithm by using `Seurat::FindClusters` function (0.15 resolution). Then, non-parametric Wilcoxon Rank Sum Test was applied on the raw counts across clusters by using `Seurat::FindAllMarkers` function to determine differentially expressed cell type markers in each cluster. These differentially expressed (Bonferroni correction was implemented, fold change > 1.20) cell markers were used to annotate the identified clusters (see Appendix C for differential testing results and see Appendix B.2 and B.3 for details of CNS annotation). Since cluster-4 was predominantly composed of cells from AD donors (Appendix B.1), it was removed prior to cluster annotation. After the annotation of major CNS cell types, astrocytes were subsetted and saved for downstream integration processes.

3.3. snRNA-seq Data Processing for the Morabito Dataset

Gene expression count matrices from the donors of the Morabito dataset (Morabito et al., 2021) (7 control, 11 AD) were introduced into R independently. The same quality control metrics as mentioned for the Sadick dataset were assessed, and filtrations, normalizations and feature selections were performed (see Appendix A.6-9 for the distribution of unfiltered & filtered quality control metrics for the Morabito dataset). The removal of the same set of transcripts mentioned in the previous section from the set of variable features led to 1995 variable genes. Different from the analysis of Sadick snRNA-seq data analysis, no integration or batch correction was implemented here. After merging the donors, PCA was implemented and it was followed by Seurat::ElbowPlot function to determine the number of principle components to be used in downstream analyses. tSNE, SNN, the Louvain algorithm and cluster annotation steps were applied as mentioned in the previous section for the Sadick dataset (see Appendix C for differential testing results), and astrocytes were subsetting and saved for downstream integration processes after the annotation of major CNS cell types (Appendix B.4 and B.5).

3.4. Integration of Astrocytes Obtained from the Sadick and Morabito Datasets by CCA

Firstly, the subsetting astrocytes from the Sadick and Morabito datasets were imported into R. All assays and slots, except the RNA assay that stores raw counts, were removed from the Seurat object. Then, the count matrices of astrocytes from each donor were independently normalized with SCTransform::SCTransform function. For each donor, 2000 variable features were selected and percentages of mitochondrial, ribosomal, and heat-shock proteins as well as hemoglobin transcripts were regressed out during this normalization step. Then, by using Seurat::SelectIntegrationFeatures function, features that will be used in the integration of donors were selected and mitochondrial, ribosomal, and heat-shock proteins as well as hemoglobin transcripts were removed among those variable features as well (1991 variable genes remained). Subsequently, the normalized data was prepared for CCA by Seurat::PrepSCTIntegration function. Integration by CCA was performed using Seurat::FindIntegrationAnchors and Seurat::IntegrateData functions respectively (dims=50 and k.weight=60). After the integration, linear (PCA) and non-linear

dimension reductions (tSNE) were performed by using the first 50 PCs. Then SNNs were computed, and the Louvain algorithm was used for clustering (0.15 resolution) leading to the formation of 9 subclusters. After the clustering, Wilcoxon rank sum test was used to identify subtype-enriched transcripts.

3.5. Differential Gene Expression Analysis for Astrocyte Subtypes

After identifying the subtypes of astrocytes, differentially expressed genes (DEGs) were determined by comparing AD and control astrocytes in each subtype independently. For the DEG analysis, DESeq2 (Love et al., 2014) and Zero-Inflated Negative Binomial-based Wanted Variation Extraction (ZINB-WaVE) (Risso et al., 2018) R packages were used in combination as our integrated snRNA-seq data was zero-inflated (see below). The DEG analysis was performed for each subtype as well as ignoring the subtype-specific information (see Appendix D for differential testing results).

For cluster-specific differential testing, cluster-specific Seurat objects were generated, and differential testing processes were conducted using cluster-specific Seurat objects, thereby, steps given below were implemented into each Seurat object independently. Firstly, the Seurat object of CCA-integrated astrocytes was converted into SingleCellExperiment (SCE) object. Genes were kept if they have a total count of at least 6 in at least 6 cells, and other genes were removed. After filtering out the lowly expressed genes, zinbwave::zinbwave function was run to extract low-dimensional signal from zero-inflated data, with the following parameters: $K=0$, $\epsilon=10^{12}$, $\text{observationalWeights}=\text{TRUE}$. Subsequently, size factors were estimated by using `scrn::computeSumFactors` function in `scrn` R package (v1.24.1) (Lun et al., 2016). Lastly, `DESeq2::DESeq` function was run with following parameters optimized for snRNA-seq data (Ahlmann-Eltze & Huber, 2020; Van den Berge et al., 2018; Zhu et al., 2019): $\text{test} = \text{LRT}$, $\text{minmu} = 10^{-6}$, $\text{minRep} = \text{Inf}$, $\text{reduced} = 1$. Genes were considered as differentially expressed if they have Benjamini-Hochberg adjusted p-value ≤ 0.05 and considered as upregulated and downregulated if $\text{FC} > 1.20$ and $\text{FC} < 0.83$ respectively.

3.6. KeyPathwayMiner and Functional Enrichment Analysis

After the identification of differentially expressed genes in each subtype between control and AD cells, these genes were used as input to KPM (List et al., 2016), which is a method to discover subnetworks in a given biological interaction network. By this network-based approach, DEGs were mapped onto a human protein-protein (PPI) interaction network. The human PPI network (version 4.4.222) was downloaded from the BioGRID database. Only the physical interactions and human-human based interactions were kept in network. Prediction based interactions and any non-human interactions were removed from the network, leading to 18,926 proteins and 1,065,083 interactions. KPM was run for each subtype (see Appendix D network-based analysis results). For this study, KPM Standalone v5.1 was used with following parameters: L1 = 0, strategy = INES, algo = GREEDY, maxSolutions = 1, K = 5. Subsequently, the genes in the subnetworks discovered by KPM were subjected to functional enrichment analysis using gprofiler2 (Kolberg et al., 2020) to determine subtype specific molecular patterns and responses to AD. Gprofiler2::gost function in R was used to run the enrichment analysis. Cytoscape (Shannon et al., 2003) was used for the visualization of interactions.

3.7. snATAC-seq Data Processing and Reclustering of ATAC-seq Astrocytes

The peak matrix of the Morabito snATAC-seq dataset (Morabito et al., 2021) was downloaded and introduced into R environment. Total number of peaks, nucleosomal signal and TSS (transcriptional start site) signal are commonly used quality control metrics for snATAC-seq data. These metrics were assessed and cells in donors were filtered based on these metrics as Seurat recommended (see Appendix A.10-15 for the distribution of unfiltered & filtered quality control metrics for the Morabito snATAC-seq dataset). For normalization and dimension reduction, LSI (latent semantic indexing) method was used. Signac::RunTFIDF, Signac::FindTopFeatures, Signac::RunSVD functions were run for normalization, selection of highly variable peaks and dimension reduction respectively. Signac::DepthCor and Seurat::ElbowPlot functions were used to determine the number of LSI components to be used in downstream analyses. First LSI dimension keeps technical variation due to sequencing depth most of the time, therefore, it was removed. Then, non-linear dimension

reduction method tSNE was implemented (dims = 2:15) and followed by `Seurat::FindNeighbors` and `Seurat::FindClusters` functions (0.1 resolution and the Louvain algorithm). Subsequently, LR (Logistic regression) test was implemented by using `Seurat::FindAllMarkers` function to determine the differentially accessible peaks in each cluster (see Appendix C for differential testing results). Criteria for significance of peaks were the same as above. Identified clusters were annotated (Appendix B.6 and B.7) using the differential accessibility of peaks. After the annotation of major CNS cell types, astrocytes were subsetted and reclustered. For the reclustering of ATAC-seq astrocytes, `Signac::RunTFIDF`, `Signac::FindTopFeatures` and `Signac::RunSVD` functions were run in order. Then, `Signac::DepthCor` and `Seurat::ElbowPlot` functions were used to determine how many LSI components will be used in downstream analyses. Then, non-linear dimension reduction method tSNE was implemented (dims = 2:10) and the Seurat object for the ATAC-seq astrocytes were saved for multi-modal integration.

3.8. snRNA-seq and snATAC-seq Integration

Seurat objects of both CCA-integrated RNA-seq astrocytes (42,020) and pre-processed ATAC-seq astrocytes (9,554) were introduced into R environment. Default assay of CCA-integrated astrocytes was set as RNA assay, and Seurat's fundamental pre-processing workflow (`NormalizeData`, `FindVariableFeatures`, `ScaleData` respectively) was implemented into this assay. After identifying 2000 variable features, which will be used for label transfer, mitochondrial, ribosomal, and heat-shock proteins as well as hemoglobin transcripts were removed among those variable features, and 1977 variable features remained. Then, gene activities for these variable features were calculated by using `Signac::GeneActivity` function and the calculated gene activities were added as an assay into the Seurat object of ATAC-seq astrocytes and they were log-normalized and scaled by `Seurat::NormalizeData` and `Seurat::ScaleData` respectively. Then, CCA was performed by using `Seurat::FindTransferAnchors` function (reduction = cca) to identify anchors between datasets. Afterwards, `Seurat::TransferData` function was used to transfer the cell type labels (dims=2:30). Five of the subtype labels in the RNA-seq astrocytes (cluster-0, cluster-1, cluster-2, cluster-3, cluster-6) were successfully transferred into ATAC-seq astrocytes (5632, 2618, 954, 203 and 144 cells identified for given clusters

respectively) (Appendix B.9 and B.10). In order to check the quality of the label transfer process, RNA-seq and ATAC-seq astrocytes were co-embedded (Appendix B.11). For the co-embedding, the gene expression matrix of RNA-seq astrocytes (in RNA assay) were imputed into ATAC-seq astrocytes based on the previously computed anchors. Then the snRNA-seq and snATAC-seq astrocyte Seurat objects were merged. After the merging, dataset was centered by using `Seurat::ScaleData` function. Then PCA was implemented by `Seurat::ElbowPlot` function, and 30 PCs were used in downstream analyses. After the linear dimension reduction, Harmony R package (v0.1.1) (Korsunsky et al., 2019) was used to remove batch effects across snRNA-seq and snATAC-seq datasets. After the batch correction, non-linear dimension reduction tSNE was implemented by using the Harmony embeddings (Appendix B.11). Low dimensional representation of RNA- and ATAC-seq astrocytes, prediction scores and correlation between promoter accessibility and gene expression show the accuracy of the label transfer process (Appendix B.9-11).

3.9. Differential Chromatin Accessibility, Motif and Motif Foot-Printing Analysis

For differential chromatin accessibility analysis, DESeq2 was used with the same parameters used for snRNA-seq data due to the similarities in the distributions of snRNA-seq and snATAC-seq datasets. We only used peaks that fall into the promoter regions for the differential testing. We identified 14,117 unique peaks that fall into the promoter regions, and all chromatin accessibility analyses were conducted using these peaks. After the differential testing, `Signac::ClosestFeature` function was used to annotate the peaks. The results were considered to be differentially accessible if $p\text{-value} < 0.01$ and $FC > 1.20$ or < 0.83 .

For a set of selected genes that exhibit both differential expression and differential chromatin accessibility, we conducted motif analysis to determine their potential regulators e.g. transcription factors (TFs). We focused on TFs that were found to be differentially expressed in our snRNA-seq during AD. We identified differentially expressed transcription factors in each cluster (cluster-0,1,2,3 and 6) and we implemented cluster-specific motif analysis for these transcription factors in their respective clusters. Firstly, we downloaded motif position and frequency matrices from JASPAR2022 database, and this information was added into our Seurat object. For

each subtype identified in the snATAC-seq data, we identified accessible regions by using `Signac::Accessible Peaks` function and we selected peaks that fall into the promoter regions. Then, we used `Signac::GetMotifData` function to obtain potential promoter regions that our TF of interest can bind to. Moreover, `Signac::Footprint` function was used for TF footprinting analysis, and by this way, we validated the binding of specific TFs in their respective clusters.



4. RESULTS

4.1. Integration of Astrocytes from Two Different Datasets Reveals Transcriptomic Differences Between Subtypes

Two single-nuclei datasets from post-mortem brain (Morabito et al., 2021; Sadick et al., 2022) were pre-processed and cell types were identified. Integration of astrocytes obtained from the two datasets yielded 9 transcriptomically distinct subtypes (Figure 4.1A-B). These subtypes were verified to be not driven by any batch effect such as dataset, disease state (Figure 4.1C-F), donor, sex and age (Appendix B.8). tSNE and bar plots show dataset and disease -based distributions of cells, validating proper integration of astrocytes (Figure 4.1C-F). In all subtypes, percentages of AD astrocytes were over 60%. Since the number of astrocytes obtained from the Morabito dataset was low compared to the Sadick dataset, some subtypes are predominantly represented by Sadick dataset (Figure 4.1F). In particular, 38,207 and 3813 cells obtained from Sadick and Morabito datasets respectively resulted in 42,020 astrocytes (Figure 4.1A). Among 42,020 cells, 29,153 were AD astrocytes and 12,867 were healthy astrocytes (see Appendix B.8 for details). Non-parametric Wilcoxon Rank Sum test was conducted to observe the transcriptional differences between subtypes, and genes significantly highly expressed in each subtype were identified. Such genes were termed enriched genes. Number of uniquely enriched genes in each cluster and the intersection of enriched genes between clusters were given in Appendix B.8 and Figure 4.1G respectively. Highest number of enriched genes were identified in cluster-2 and cluster-6 by 476 and 466 genes respectively. While 425 of 466 genes were uniquely enriched in cluster-6, 391 of 476 genes were uniquely enriched in cluster-2. A heatmap visualizing top 5 uniquely enriched genes in each subtype based on the fold-change value were given in Figure 4.1B. GRK3 (G protein-coupled receptor kinase 3), which was shown to be expressed by astrocytes in human AD hippocampus (Guimarães et al., 2021), was uniquely enriched in cluster-0 astrocytes. Glutamine transporter SLC38A1 (SNAT1) was previously reported to be downregulated during AD (Fan et al., 2021), and it was uniquely enriched in cluster-1 astrocytes. Glutamate release from presynaptic terminals was shown to be decreased in human AD brains, leading to impaired synaptic transmission among glutamatergic neurons (Fan et al., 2021). As glutamate-glutamine cycle is crucial for proper synaptic transmission, enriched

SLC38A1 expression in cluster-1 might be important for protective pathology. VIM (Vimentin) and SYNM (Synemin) are intermediate filament proteins and contribute to astrocyte cytoskeleton, and they are uniquely enriched in cluster-2 and cluster-3 respectively. SERPINA3 was found to be upregulated in human frontal cortex samples in early AD and it was shown to be predominantly produced by reactive astrocytes, which surrounds the senile plaques (Vanni et al., 2017a; Zattoni et al., 2022a). SERPINA3 was found to be uniquely enriched in cluster-3 astrocytes and might be contributing to AD pathology. APOE (apolipoprotein E), major risk factor gene for idiopathic late-onset AD, was enriched in cluster-2 and cluster-4. Similarly, APP (Amyloid precursor protein) is uniquely enriched in cluster-6. Apart from the uniquely enriched transcripts, there are also similarities across subtypes. The highest number of commonly enriched genes was detected between cluster-2 and cluster-4 (75 genes), followed by 33 genes between cluster-4 and cluster-5. Moreover, 12 genes were commonly detected between cluster-0,4,5 and 11 genes were commonly detected between cluster-0,4,8.

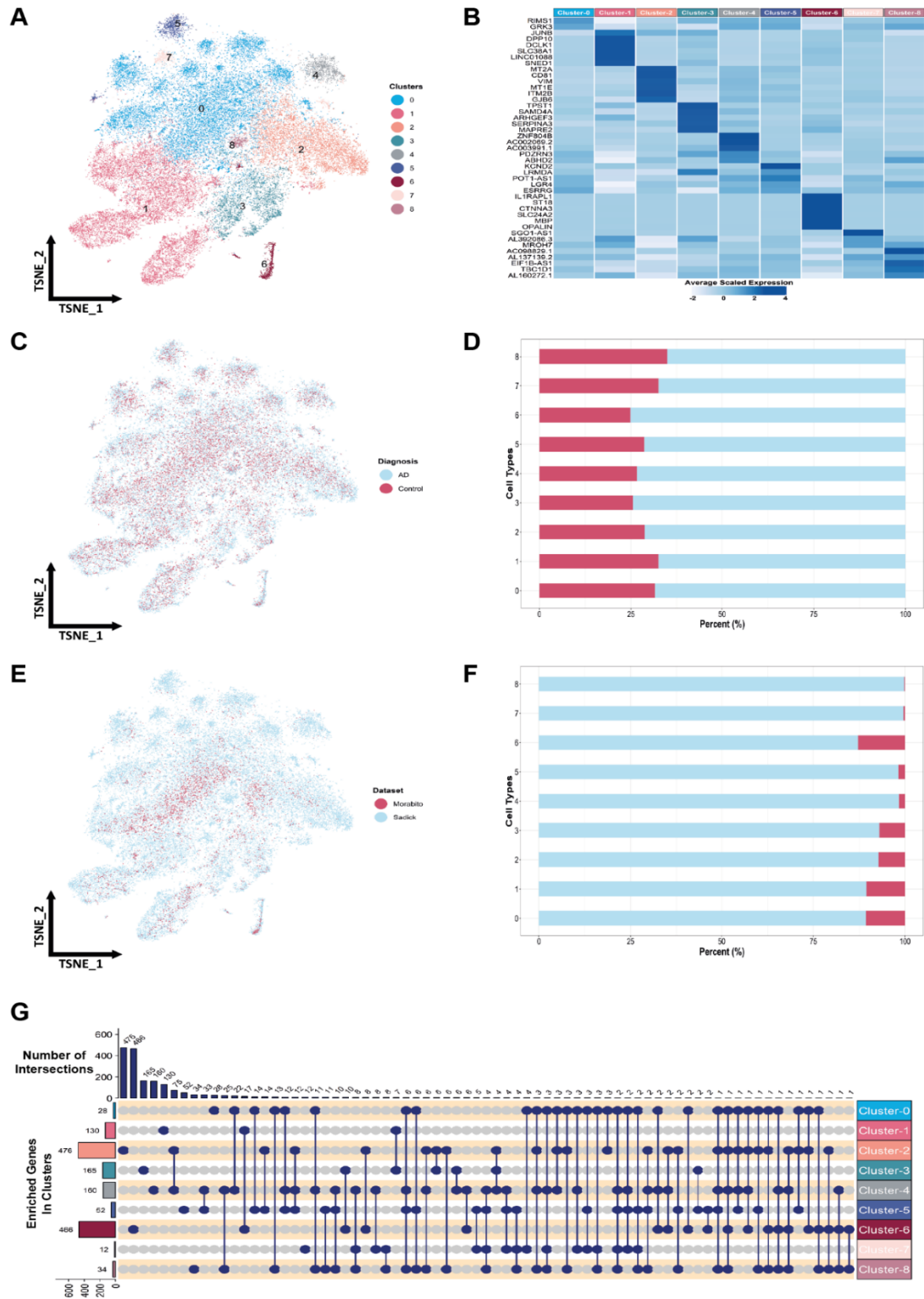


Figure 4.1: Integration of astrocytes from two different datasets reveals transcriptomic differences between subtypes. (A) tSNE plot of astrocyte subtypes (42,020 nuclei). (B) Average scaled expression of uniquely enriched genes in astrocyte subtypes. (C, D) Diagnosis- and (E, F) dataset-based distribution of astrocyte subtypes. (G) Upset diagram of intersection of enriched genes in astrocyte subtypes.

4.2. Single-nuclei Astrocyte Integration Followed by Mapping DEGs onto Human PPI Network Was Proven to Capture AD-Related Genes More Sensitive Compared to Traditional Approaches

Differential gene expression analyses conducted to compare AD and control astrocytes in each cluster revealed upregulated and downregulated genes during AD. These genes were mapped on the human protein-protein interaction network using KeyPathwayMiner, and subnetworks with differentially up/down regulated and physically-interacting genes were discovered. Number of up and downregulated elements (differentially expressed genes and subnetwork genes) for each subtype during AD were given Table 4.1. Additionally, a comprehensive AD-related gene list was generated by Elif Düz (unpublished), which was composed of 772 genes, by using the combination of a set of genes obtained from GeneCards, Gene Ontology and KEGG databases and Hu et al., 2017 study were used in this study. Then, AD-related genes in our clusters were also identified (see Table 4.1). Moreover, all AD and all healthy astrocytes were also compared by ignoring subtypes (referred as subtype-unaware comparison) to validate the benefit of subtype specific approach.

Cluster-2 contains the highest number of AD-related genes (55) although the highest number of differentially expressed genes were obtained in Cluster-3 (875). Similarly, highest number of AD-related upregulated and downregulated genes were obtained in Cluster-2 (45) and Cluster-3 (20) respectively. The least number of AD-related genes were detected in Cluster-8 and Cluster-7.

By “subtype-unaware” comparison of AD and control astrocytes, 51 differentially expressed AD-related genes were identified in total, 45 of which were available in the discovered subnetwork. On the other hand, 107 unique AD-related genes were obtained from the combination of all subtype-specific results, and 94 of them were the part of subtype-specific subnetworks. 69 AD-related genes were only identified in the subtype-specific differential gene expression analysis, and this reveals the importance of our subtype-specific approach. Our single-nuclei dataset integration followed by mapping differentially expressed genes on human PPI network was proven to capture AD-related genes more sensitive compared to the original study that generated the data (Sadick et al., 2022) where they also conducted differential gene expression analysis for the 9 astrocyte subtypes and identified 65 unique AD-related genes.

Table 4.1: Number of differentially expressed elements in astrocyte subtypes. DGEA column shows the number of differentially expressed genes and KPM column shows the number of subnetwork genes obtained after mapping differentially expressed genes onto human PPI network. Number of AD related genes identified in each gene set is also given. (DGEA: Differentially Gene Expression Analysis; KPM: KeyPathwayMiner; AD-Rel.: Alzheimer's disease related genes)

Clusters	DGEA			KPM		
	Up	Down	AD-Rel.	Up	Down	AD-Rel.
Cluster-0	458	275	32	299	137	26
Cluster-1	470	342	44	334	181	36
Cluster-2	542	211	55	406	109	48
Cluster-3	449	426	41	310	296	32
Cluster-4	282	162	20	212	91	17
Cluster-5	233	135	19	172	84	15
Cluster-6	322	243	29	244	146	20
Cluster-7	175	127	10	132	78	8
Cluster-8	144	125	9	103	72	7
Subtype-unaware	723	394	51	485	185	45

KeyPathwayMiner is a *de novo* pathway enrichment method to find the pathways within a biological network. Our aim was to map differentially expressed genes onto human Protein-protein interaction network to create a subnetwork with more biologically relevant genes since this approach filters the list of DEGs such that those forming a physically-interacting subnetwork are reported. Largest and smallest subnetworks were obtained in Cluster-3 and Cluster-8 respectively. Subnetwork genes were subjected to gene ontology analysis for the identification of subtype-specific altered functions in AD.

4.3. Subtype-specific Altered Biological Processes and Molecular Functions were Identified by Gene Ontology Analyses

By using the up and downregulated subnetwork genes in each cluster, gene ontology analyses were conducted, and altered biological processes and molecular functions were identified. Identified GO-terms were classified based on their involvement in certain processes like AD pathology, astrocyte process, cell death, fatty acid and lipid metabolism, immune response, ion metabolism and mitochondria (Figure 4.2). We found that, apoptosis related GO-terms were upregulated in AD astrocytes in all clusters. Apoptotic process, cell death, regulation of apoptotic process and regulation of cell death were found to be upregulated nearly in all clusters. Interestingly, we didn't obtain any apoptosis related GO-terms in overall AD astrocyte phenotype (e.g. subtype-unaware) despite their abundances in subtype specific results. Cognitive impairment is one of the devastating results of AD and we identified the cognition term to be downregulated in cluster-3 and cluster-6 AD astrocytes. Moreover, learning or memory term was uniquely downregulated in cluster-3 AD astrocytes. Response to oxidative stress term was found to be upregulated in AD astrocytes in cluster-0,1,2,5 and overall AD astrocyte phenotype. However, in cluster-3 AD astrocytes, it has both upregulated and downregulated components. Similarly, response to reactive oxygen species term was found to be upregulated in cluster-2 but downregulated in cluster-3 AD astrocytes. Oxidative stress might enhance the disease pathology, thereby, oxidative stress response dysregulation/downregulation in cluster-3 AD astrocytes might be neurotoxic. The fact that these oxidative stress related terms were found to be upregulated in overall AD astrocyte phenotype strengthens subtype specific neurotoxic contributions to AD. Astrocytes can directly be involved in A β and NFT formation (Chiarini et al., 2017). We found that AD pathology related GO-terms such as amyloid beta metabolic process, amyloid beta formation, amyloid precursor protein catabolic process, regulation of amyloid-beta formation, tau protein binding, amyloid precursor protein metabolic process, negative regulation of amyloid-beta formation terms were uniquely upregulated in cluster-6 AD astrocytes. Also, the positive regulation of tau-protein kinase activity was found to be upregulated in cluster-4,5 and 6 AD astrocytes.

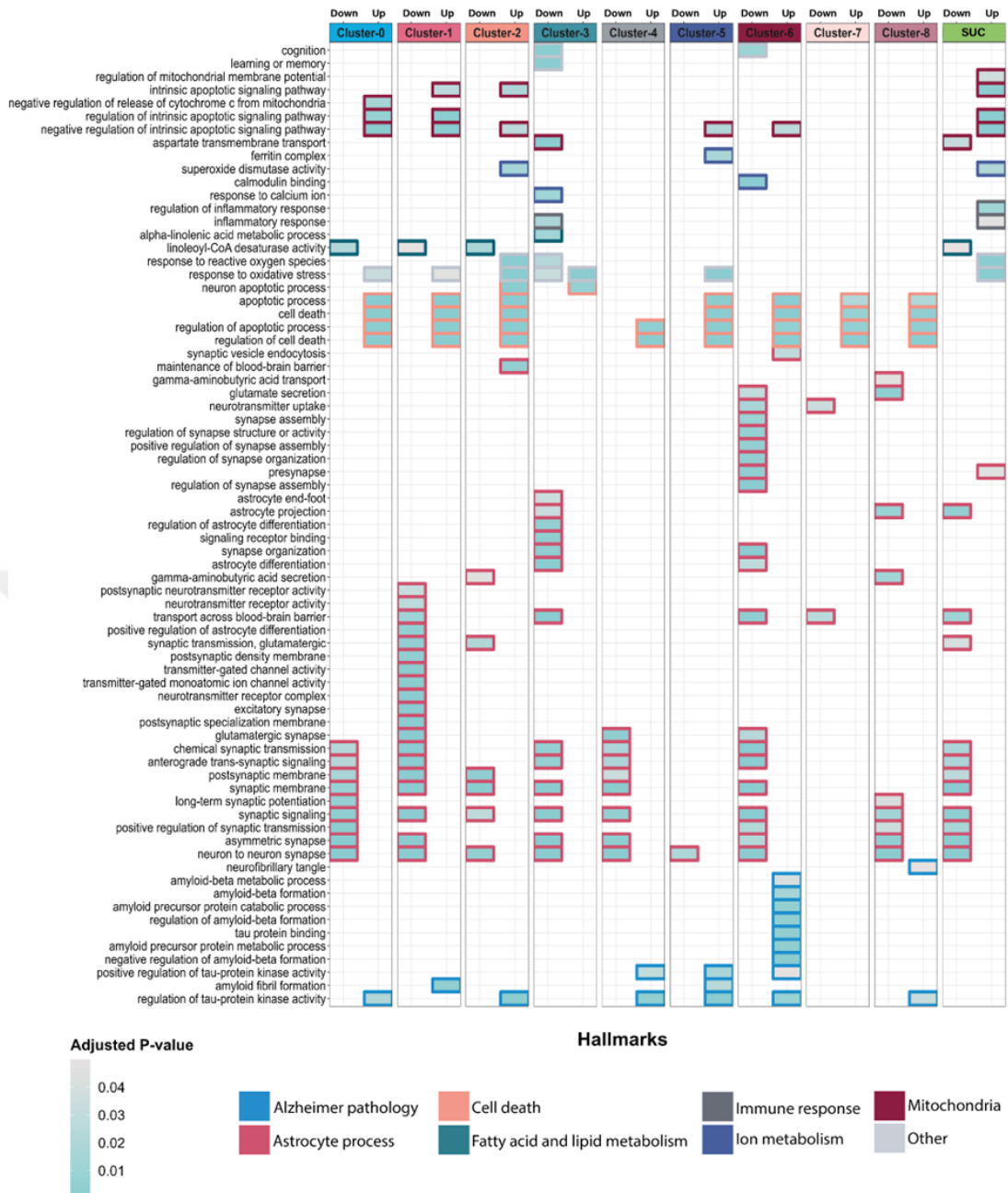


Figure 4.2: Altered biological processes in astrocyte subtypes during AD. Certain biological processes and functions were found to be altered in astrocyte subtypes during AD. Due to change in many cellular processes, these changes were grouped into several hallmarks. Hallmarks are colour-coded in their frameworks and colours inside the rectangles change based on the adjusted p-value (Benjamini Hochberg corrected) in their respective gene ontology analysis. SUC: Subtype-unaware comparison.

In addition, neurofibrillary tangle term was found to be uniquely upregulated in cluster-8 AD astrocytes. Cluster-6 has the highest number of AD pathology related terms (APP, A β and NFT-related). Aspartate transmembrane transport term was found

to be uniquely downregulated in cluster-3 AD astrocytes and this situation is also valid for overall AD astrocyte phenotype. In addition, inflammatory response term was found to be downregulated in cluster-3 AD astrocytes but upregulated in overall AD astrocyte phenotype. This situation also reveals the contrast between overall phenotype and cluster-3. Moreover, alpha-linolenic acid metabolic process term was found to be uniquely downregulated in cluster-3 AD astrocytes. A recent study revealed that alpha-linolenic acid contributes to AD pathology by regulating the susceptibility of BBB integrity (Leikin-Frenkel et al., 2022). There are also other uniquely identified terms such as maintenance of blood brain barrier, which was found to be uniquely upregulated in cluster-2 AD astrocytes and can be neuroprotective. Moreover, ferritin complex term was found to be uniquely upregulated in cluster-5 AD astrocytes. This situation might be associated with iron storage, and hence contribute to AD pathology. While synaptic signalling related GO-terms were downregulated in almost all clusters, synapse structure, assembly and organization related GO-terms were uniquely downregulated in cluster-6 AD astrocytes. We identified heterogeneity across subtypes in AD through GO analysis. For further characterization, we discussed up and downregulated subnetwork genes in AD landscape.

4.4. Apoptosis and Autophagy Perspective in Astrocyte Subtypes During AD

It is a well-known fact that A β leads to neuronal death and astrocyte activation and various mechanisms have been proposed regarding how A β leads to neuronal cell death. However, considering massive impact of astrocytes on neuronal functioning, maintenance and survival, an important question is how astrocytic cell death is regulated in AD. Pro-Apoptotic WT1 Regulator (PAWR, or PAR-4) was found to regulate apoptosis of astrocytes (G. Wang et al., 2012) and recently, it was also found that, amyloid peptide can trigger apoptosis in primary astrocytes through PAR-4 in vitro (G. Wang et al., 2012). Moreover, PAR-4 was found to be expressed by apoptotic astrocytes that surround amyloid plaques (G. Wang et al., 2012) and we found that PAR-4 was uniquely upregulated in cluster-1 AD astrocytes, and they might be closely associated with amyloid plaques. Upregulation of PAR-4 might be associated with the apoptotic roles of cluster-1 AD astrocytes, and it might be crucial for disease

progression. Thereby, amyloid plaques might be associated with the astrocytic cell fate.

Many different cellular mechanisms might be involved in the prevention of the accumulation of proteins in AD, and failure of these mechanisms might also affect the onset of AD. Autophagy is one of the waste management system in cells for degradation of proteins and organelles and has recently become a hot topic for many diseases including AD. Autophagy is strongly associated with oxidative stress, inflammation and aging, and these factors are also strongly associated with AD. Astrocyte-mediated autophagy in AD have not been completely enlightened but oxidative stress have been proposed to induce autophagy in astrocytes (J. L. Wang & Xu, 2020). Moreover, Peroxisome Proliferator Activated Receptor Alpha (PPARA) was hypothesized to be involved in autophagy in CNS and related to AD pathology (Luo et al., 2020). PPARA agonists (gemfibrozil and Wyl4643), which activate PPARA, were shown to improve autophagy in astrocytes and improves memory impairment in mice (Luo et al., 2020). PPARA agonists also led to recruitment of astrocytes near amyloid plaques and led to enhanced clearance of plaques (Luo et al., 2020). PPARA was found to be uniquely upregulated in cluster-8 AD astrocytes. Therefore, those astrocytes might be acting as neuroprotective.

4.5. Cholesterol Production and Lipid Metabolism in Astrocyte Subtypes During AD

In recent years, there has been intensive research into the role of impaired cholesterol metabolism in AD pathogenesis. Excess of cholesterol in brain cannot pass from BBB. For this reason, it is converted into oxysterols which are capable of passing through BBB and leaving brain (Marwarha & Ghribi, 2015). But due to BBB disruption in AD, oxysterols can accumulate in brain and subsequently exacerbates oxidative stress and inflammation (Dias et al., 2014). Thereby, cholesterol metabolism and regulation of cholesterol levels are highly associated with AD pathology. Astrocytes are responsible for cholesterol production and delivery (Staurengi et al., 2021) and cholesterol is also required for oligodendrocyte survival and myelin production (Molina-Gonzalez et al., 2023) thereby, overall maintenance of CNS. Farnesyl diphosphate synthase (FDPS) gene encodes for an enzyme that catalyses the production of farnesyl pyrophosphate, which is an intermediate product in cholesterol synthesis (Wollmer, 2010). FDPS was

found to be uniquely downregulated in cluster-1 AD astrocytes. Thereby, regulation of FDPS in cluster-1 might affect cholesterol levels in CNS and can be crucial for AD pathology.

In the brain, cholesterol is formed and binds with ApoE, a protein produced by astrocytes, to create lipoproteins. These lipoproteins are then transferred into neurons by ABC transporters. Among these transporters, ABCA1 plays a vital role in maintaining stable levels of cholesterol in the brain. It achieves this by controlling the amount of ApoE through the transfer of cholesterol to unlipidated ApoE (Staurenghi et al., 2021). Therefore, ABCA1 regulates ApoE levels. Additionally, it was shown in mice that, enhanced ABCA1 activity rescued impaired A β degradation (Rawat et al., 2019). ABCA1 was found to be upregulated in all AD astrocyte clusters, and overall AD astrocyte phenotype, except for cluster-3 and cluster-4 AD astrocytes. Upregulation of ABCA1 in given clusters might be associated with neuroprotective behaviours.

Low-density lipoprotein receptor (LDLR) is one of the APOE receptors and expressed by astrocytes (Rebeck et al., 1993). LDLR controls the abundance of ApoE in brain and, by this way, it controls the uptake and degradation of A β by astrocytes (Basak et al., 2012). Increased LDLR levels were shown to decrease apoE levels and A β accumulation in APP/PS1 mice (J. Kim et al., 2009). Also, in another study, increased LDLR was shown to enhance the degradation of A β (Basak et al., 2012). Moreover, A β was also found to be directly bind to LDLR receptors, therefore, astrocytes significantly contribute to A β uptake and clearance by their LDLR receptors (Basak et al., 2012). LDLR was found to be uniquely downregulated in cluster-3 AD astrocytes and this might be reducing the astrocytic A β uptake and clearance and thereby, might be considered as neurotoxic.

Clusterin (CLU) is a well-known LOAD gene related with cholesterol, and it has been reported that Clu overexpression in 5xFAD mice astrocytes resulted in reduced A β pathology (Chen et al., 2021). CLU was previously reported to be upregulated in human brain prefrontal cortex AD astrocytes (Mathys et al., 2019). Consistent with previous findings, CLU was found to be upregulated in all AD astrocytes and overall AD astrocyte phenotype, except for cluster-3. Therefore, upregulation of CLU in all clusters, except for cluster-3, might be considered as neuroprotective behaviour.

4.6. A β Interacts with Astrocytic Immune Modulators

Astrocytes and microglia are important residents for CNS and regulate immune responses. Toll-like receptor 4 (TLR4) gene is critical component of innate immune system in CNS, and it can be found in different cell types including astrocytes, microglia, and oligodendrocytes (Bowman et al., 2003; Olson & Miller, 2004). Activation of TLR receptors triggers NF κ B signalling and leads to the activation of inflammation-related genes like TNF- α , IL-6, IL-1 β , iNOS etc. (Zhou et al., 2020). During AD, A β can bind to TLR4 and trigger microglia activation (Wu et al., 2022). Activated microglia can later lead to the release of proinflammatory cytokines (IL-6, IL-1 β , TNF- α), which might subsequently lead to astrocyte activation (Wu et al., 2022). A β was also shown to bind astrocytic TLR4, and its activation was shown to improve the clearance of amyloid plaques through enhanced phagocytosis capacity of glial cells in AD mouse models (Y. He et al., 2020; Qin et al., 2016; Tahara et al., 2006; Wang et al., 2018). TLR4 was found to be uniquely downregulated in cluster-1 AD astrocytes. However, there is no known correlation between TLR4 expression and enhanced inflammatory response in astrocytes (Li et al., 2021). TLR4 downregulation in cluster-1 AD astrocytes might be caused by the internalization of this receptor due to high exposure to A β , therefore, it is hard to classify this response as neuroprotective or neurotoxic. Therefore, cell surface elements of astrocytes might be important therapeutic targets for AD.

CD44 is a cell surface glycoprotein involved in inflammation and can be found in astrocytes (Akiyama et al., 1993; Pinner et al., 2017). The number of CD44 positive astrocytes was shown to be increased in the brains of patients with AD (Akiyama et al., 1993). Moreover, it was also found that reactive astrocytes that surround the amyloid plaques were always labelled as CD44 positive (Akiyama et al., 1993). CD44 was also found to be upregulated in entorhinal cortex AD astrocytes and considered to be as a pan-reactive astrocyte marker (Leng et al., 2021). CD44 was found to be upregulated in cluster-0,1,2,4,6,8 AD astrocytes and over all AD astrocyte phenotype. As it was found to be closely associated with astrocytes that surround amyloid plaques, its impact on AD pathology needs to be further characterized.

Similar to CD44, Guanylate Binding Protein 2 (GBP2) was considered to be an inflammatory reactive astrocyte marker (Liddel et al., 2017) and it has been shown

that astrocytic GBP2 expression was higher in human AD brain compared to healthy cases (H. Kim et al., 2022). GBP2 was found to be uniquely upregulated in cluster-1 AD astrocytes and overall AD astrocyte phenotype. Since inflammatory reactive astrocytes have been proposed to secrete cytokines that will increase the burden of neuroinflammation (H. Kim et al., 2022), upregulation of GBP2 in cluster-1 might be neurotoxic.

Another reactive astrocyte related gene is Serpin Family A Member 3 (SERPINA3 or ACT), and it is a protease inhibitor that is expressed by astrocytes in CNS and involved in inflammation and apoptosis (Zattoni et al., 2022b). Further studies have shown that SERPINA3 is mainly expressed by reactive astrocytes that surround amyloid plaques (Abraham, 2001) and it was found to be upregulated in frontal cortex of patients with AD (Vanni et al., 2017b). Also, it has been shown that A β binds with SERPINA3 in vitro (Abraham, 2001) and overexpression of SERPINA3 has been proposed to promote A β accumulation (Abraham, 2001). SERPINA3 was found to be upregulated in cluster-0,2,4,5 and 6 AD astrocytes, therefore, those clusters might be promoting the A β accumulation.

Another crucial signalling pathway is Phosphatidylinositol 3-kinase (PI3K, or PIK3CA) and it is involved in inflammation, maintenance of synaptic plasticity and associated with long-term potentiation which was shown to be associated with memory (Horwood et al., 2006; Tahirovic & Bradke, 2009). Recent studies show that Notch-PI3K-AKT signalling might regulate reactive astrocytes and their cytokine production under inflammatory conditions (Cheng et al., 2020). It was found that, in reactive astrocytes (generated by LPS+IFN γ stimulation), PI3K and AKT levels were increased, and this indicates that PI3K-AKT signalling pathway is activated in reactive astrocytes (Cheng et al., 2020). Similarly, in our results, PI3K was found to be upregulated in cluster-2,3 and 5 AD astrocytes. Additionally, AKT3 was found to be uniquely upregulated in cluster-3 AD astrocytes.

4.7. Ion Overload and Disruption of Signalling Pathways in Astrocyte Subtypes In AD

Astrocytes are important contributors of CNS maintenance and neuronal survival. One of their roles in CNS is the regulation of ion metabolism. Solute Carrier Family 39 Member 14 (SLC39A14) is a transmembrane transporter expressed by astrocytes that

regulates the uptake of iron, zinc and manganese (Jenkitkasemwong et al., 2018; Routhe et al., 2020). Iron is an important molecule for CNS maintenance as it is directly required for myelin formation, thereby also associated with oligodendrocytes. Astrocytes, however, are responsible for iron transport as they are strongly associated with blood vessels. Previously, SLC39A14 expression was shown to be increased in astrocytes in response to inflammation and was reported to regulate iron uptake, which might contribute to AD pathology, as iron deposition is a widespread condition observed in many neurodegenerative disorders (Routhe et al., 2020). Iron uptake (and SLC39A14 expression) was shown to be increased in IL-1 β treated astrocytes (Routhe et al., 2020). Thereby, astrocytes seem to increase their iron uptake in inflammatory conditions. Upregulation of iron uptake regulators might lead to iron accumulation in reactive astrocytes, and reactive astrocytes were shown to have high iron uptake compared to resting astrocytes (Cheli et al., 2020; Pelizzoni et al., 2013). SLC39A14 was found to be uniquely upregulated in cluster-1, and this may be considered as neurotoxic as it might lead to iron overload.

Similar to iron overload, calcium signalling, and calcium overload have been reported to be crucial in AD. Phospholipase C- β 1 (PLCB1) gene encodes for an enzyme that regulates calcium metabolism and have been proposed to contribute to AD pathology through calcium overload (Park et al., 2022). It was found that A β 42 oligomer treatment increased calcium release in SH-SY5Y cells, leading to calcium overload (Park et al., 2022). In parallel, PLCB1 expression was upregulated in A β 42-treated cells, and the knockdown alleviated the overload in vitro (Park et al., 2022). Therefore, calcium overload is controlled by PLCB1 and we found that PLCB1 is downregulated in all AD astrocyte clusters except for cluster-0,3 and 8. Downregulation of PLCB1 in certain astrocyte clusters might lead to the alleviation of calcium overload, thereby, might be neuroprotective. S100 Calcium Binding Protein A6 (S100A6) is a calcium binding protein found in astrocytes. During AD, it is highly expressed by astrocytes around amyloid plaques (Tian et al., 2019). It was found that, S100A6 produced by astrocytes can lead to A β degradation through competing with zinc ions (Tian et al., 2019). S100A6 was found to be upregulated in cluster-1,2 and 3 AD astrocytes and overall AD astrocyte phenotype. Therefore, the upregulation of S100A6 in these clusters might be considered as a neuroprotective behaviour. Inositol 1,4,5-Trisphosphate Receptor Type 1 (ITPR1) is another gene related with calcium

signalling pathway and can contribute to AD pathology (Gambardella et al., 2020). ITPR1 gene was found to be uniquely downregulated in cluster-3 AD astrocytes. Contribution of calcium signalling pathway to AD pathology is undeniable and astrocytic downregulation of ITPR1 might be important for disease pathology.

4.8. Neuroprotective Roles of Chaperons in Astrocyte Subtypes In AD

Chaperons are important elements for proper protein folding and they might have important contributions to AD onset and pathology. Heat Shock Protein 90 Alpha Family Class A Member 1 (HSP90AA1) and Heat Shock Protein 90 Alpha Family Class B Member 1 (HSP90AB1) are classes of HSP90 chaperons and associated with unfolded protein binding (Gonzalez-rodriguez et al., 2022). HSP90AA1 and HSP90AB1 expressions were found to be downregulated in hippocampus during AD (Gonzalez-rodriguez et al., 2022). While HSP90AB1 is expressed by healthy and AD astrocytes, HSP90AA1 is expressed by AD astrocytes, not in healthy ones (Gonzalez-rodriguez et al., 2022). HSP90AA1 was found to be accumulated inside the plaques in human hippocampus during AD, where HSP90AB1 is expressed by astrocytes surrounding plaques (Gonzalez-rodriguez et al., 2022). HSP90AA1 was found to be upregulated in AD astrocytes in all clusters and in overall AD astrocyte phenotype while HSP90AB1 was found to be upregulated in cluster-0,1,2,4,5,6 AD astrocytes and overall AD astrocyte phenotype. As chaperons are associated with protein folding, their upregulation might be related with neuroprotection. ITM2B and ITM2C were previously reported to be upregulated in human reactive astrocytes in AD (Dai et al., 2023). They also encode chaperone protein that was previously shown to decrease A β formation in human AD brain (Dolfe et al., 2018). We found that both ITM2B and ITM2C were upregulated in cluster-2 AD astrocytes. Therefore, their ability to limit A β accumulation might be neuroprotective.

4.9. Subtype Specific Responses to Oxidative Stress and Involvement of Antioxidants In AD

As brain is a metabolically active organ, many possible harmful byproducts are generated by oxidative phosphorylation. Conversion of these byproducts to less harmful metabolites is essential and have been proposed to associate with many

neurodegenerative disorders including AD. Superoxide dismutase 2 (SOD2) is an antioxidant enzyme that catalyse the conversion of superoxide into hydrogen peroxide (Murphy, 2009). SOD2 expression was observed to increase in AD patients (De Leo et al., 1998). Upregulation of SOD2 was proposed to be a useful response against high oxidative stress. Additionally, SOD2 overexpression was shown to reduce amyloid plaques (Massaad et al., 2009.). SOD2 was shown to be upregulated in cluster-0,1,2,3,4,5,6 AD astrocytes and overall AD astrocyte phenotype. For this reason, SOD2 upregulation in given clusters might be considered as a neuroprotective behaviour.

MT2A (or MT2, Metallothionein-2) is another antioxidant molecule produced by astrocytes and considered as a neuroprotective response of astrocytes against oxidative stress (Waller et al., 2018). In Cognitive Function and Ageing Study (CFAS), which contained 78 human post-mortem temporal cortex cases with different levels of brain pathology, it was found that MT2A expression in human temporal cortex astrocytes does not correlate with AD pathology, instead, it is associated with astrocyte oxidative DNA damage (Waller et al., 2018). MT2A was found to be uniquely enriched in cluster-2 and it was found to be significantly upregulated in cluster-0,4,5,7,8 AD astrocytes. Therefore, although MT2A gene expression does not exhibit correlation with AD pathology in human temporal cortex astrocytes, it was found to be associated with AD in human prefrontal cortex astrocytes and astrocyte subtypes.

NAD(P)H Quinone Dehydrogenase 1 (NQO1) is an enzyme that was proposed to be an antioxidant (SantaCruz et al., 2004). It was found that NQO1 was expressed by astrocytes that surround amyloid plaques and the upregulation of NQO1 have been proposed to be neuroprotective (SantaCruz et al., 2004). NQO1 is also a part of the nuclear factor erythroid 2–related factor 2 (NRF2) system, which is associated with defence against mitochondrial oxidative stress, and NQO1 was also found to be increased in tau-exposed astrocytes (Ungerleider et al., 2021). We also found that NQO1 was upregulated in cluster-0,2 and 3 AD astrocytes and overall AD astrocyte phenotype. Thereby, NQO1 expression can be considered as a neuroprotective behaviour.

4.10. Reduced Neurotransmitter Homeostasis in AD Astrocyte Subtypes

Genes that are related with glutamate and GABA neurotransmitters such as GLU, SLC1A2, SLC1A3 and SLC6A11 are found to be downregulated in astrocyte clusters and downregulation of these genes might be referring to reduced homeostasis in CNS, implying neurotoxicity. Specifically, GLUL was uniquely downregulated in cluster-3 AD astrocytes. Interestingly, GLUL was previously reported to be upregulated in prefrontal cortex AD astrocytes (Mathys et al., 2019) but downregulated in entorhinal cortex AD astrocytes (Leng et al., 2021). GLUL downregulation did not appear on overall AD astrocyte phenotype in our results. Similar to GLUL, SLC1A2 was found to be uniquely downregulated in cluster-3 AD astrocytes and similar results were also obtained for entorhinal cortex AD astrocytes (Leng et al., 2021), but SLC1A2 downregulation was observed in overall AD astrocyte phenotype. Of note, SLC1A2 was found to be downregulated in AD astrocytes in all clusters and overall AD astrocyte phenotype based on DGEA results. Additionally, SLC1A3 was found to be uniquely downregulated in all AD astrocytes in all clusters and overall AD astrocyte phenotype. SLC1A3 was also previously reported to be downregulated in human entorhinal cortex AD astrocytes (Leng et al., 2021). Moreover, SLC6A11 did not appear in subnetwork results but found to be uniquely downregulated in all AD astrocytes in all clusters and overall AD astrocyte phenotype. Similar to SLC1A2 and SLC1A3, it was reported to be downregulated in entorhinal cortex AD astrocytes (Leng et al., 2021).

4.11. Astrocytic Elements Regulate A β and Tau Accumulation In AD

Astrocytes were shown to produce enzymes that can degrade A β into smaller fragments and might reduce the amyloid burden in brain. One of these enzymes is called Endothelin Converting Enzyme 1 (ECE1), which is expressed by astrocytes (Nakagomi et al., 2000) and reported to be involved in the degradation of monomeric A β species (Eckman et al., 2001, 2003). ECE1 was found to be upregulated in cluster-0,1,3,5 AD astrocytes and overall AD astrocyte phenotype. Since it is involved in A β degradation, the upregulation of ECE1 might considered as neuroprotective behaviour.

In addition, elements that are associated with beta and gamma secretases, which are involved in the processing of A β from APP, are also potentially linked with AD pathology. To illustrate, Transmembrane P24 Trafficking Protein 10 (TMED10, or TMP21 or P23) is a transmembrane protein where its expression was shown to increase A β production (Shin et al., 2019). TMED10 was found to be associated with γ -secretase complex, which is important for APP processing in AD (Chen et al., 2006). Decreased TMED10 expression leads to increased γ -secretase cleavage of APP (Vetrivel et al., 2007). TMED10 was found to be uniquely downregulated in cluster-3 AD astrocytes. Therefore, it might be contributing to increased amyloid burden in AD brain.

There are also some molecules that regulate the oligomerization of A β . For example, Cystatin-C (CST3) is a cysteine protease inhibitor and have been reported to act as neuroprotective in AD as it inhibits the oligomerization of A β (Kaur & Levy, 2012). CST3 was found to be uniquely downregulated in cluster-8 AD astrocytes and its downregulation might be corresponding to the loss of protective functions.

On the other hand, Mitogen-Activated Protein Kinase 10 (MAPK10, or JNK3) is a kinase that is expressed by astrocytes (Nakano et al., 2020), and it was found to be highly expressed in human AD brain (Gourmaud et al., 2015). MAPK10 was found to enhance the production of A β and lead to the formation of NFTs (Yarza et al., 2016). As it might lead to increased amyloid and tau burden in brain, it has been proposed to be a therapeutic target for AD (Yarza et al., 2016). We found that MAPK10 is uniquely upregulated in cluster-3 AD astrocytes and might be increasing the neurotoxicity in AD.

Hyperphosphorylated tau protein accumulation that generates the NFTs is one of the hallmarks of AD, together with amyloid plaques. In AD, intraneuronal accumulation of tau protein is the well-known phenomenon, but, microtubule associated protein tau (MAPT) was shown to be also expressed by astrocytes (Chiarini et al., 2017). It was shown that A β binds to calcium sensing receptor (CASR) in human astrocytes, leading to the production and release of phosphorylated tau (Chiarini et al., 2017). Thereby, astrocytes that encounter with A β might produce hyperphosphorylated tau and release it. It was found that MAPT was upregulated in cluster-3,6 and 8 AD astrocytes and this behaviour might be considered as neurotoxic.

Another element involved in amyloid beta clearance is Aquaporin 4 (AQP4), which is an important regulator of glymphatic system and involved in important events in astrocytes including, calcium signalling, potassium balance, neuroinflammation, synaptic plasticity and have been reported to be also involved in amyloid beta and tau clearance (Silva et al., 2021; Valenza et al., 2020; Yang et al., 2012). It is mainly expressed by perivascular end-feet of astrocytes (Agre et al., 2002). Aqp4 deletion was shown to increase amyloid accumulation in 5xFAD mice cerebral cortex (Smith et al., 2019). AQP4 was found to be uniquely upregulated in cluster-2 AD astrocytes based on DGEA analysis. Surprisingly, it did not appear to be differentially up or downregulated in our network-based results. Upregulation of AQP4 in cluster-2 AD astrocytes might be considered as a neuroprotective response.

4.12. Human A1 Neurotoxic Reactive Astrocytes Were Identified in Cluster-3

In 2017, Liddelow and colleagues (Liddelow et al., 2017) reported two types of reactive astrocytes called A1 and A2, and they were generated by neuroinflammation and ischemia respectively. A1 reactive astrocytes were shown to upregulate many different sets of destructive genes, and, therefore, they were considered to be neurotoxic. On the other hand, A2 reactive astrocytes were reported to be neuroprotective as they upregulate neurotrophic factor genes. Complement component 3 (C3) gene was reported to be a marker for A1 reactive astrocytes, and they were detected in post-mortem human AD astrocytes (Liddelow et al., 2017). We found that C3 was uniquely enriched in cluster-3 (Figure 4.3A). We also found that the majority of C3⁺ astrocytes were coming from AD patients (Figure 4.3B). C3 was found to be significantly upregulated in cluster-3 AD astrocytes. Despite the fact that C3⁺ astrocytes were mainly obtained from AD patients, there are some C3⁺ astrocytes also obtained from healthy cases. Studies conducted in 2018 (Boisvert et al., 2018; Clarke et al., 2018) revealed that increase in the number of C3⁺ astrocytes is also possible with aging, thereby, validating our results.

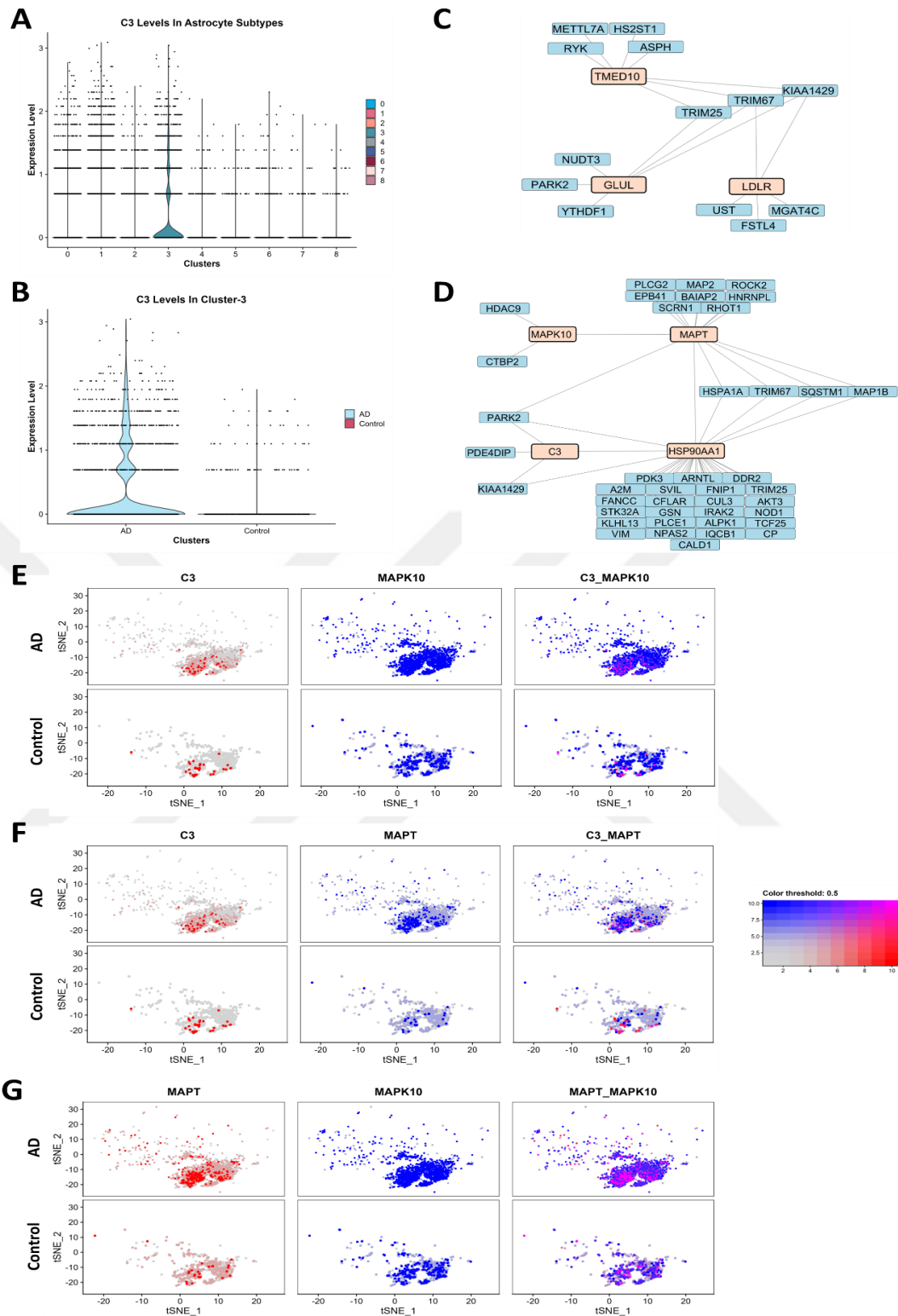


Figure 4.3: A1 neurotoxic astrocytes were found in Cluster-3, and they might increase the NFT burden in AD. (A) C3 expression across all astrocyte clusters. (B) C3 expression in cluster-3 AD and control astrocytes. (C) Cluster-3 downregulated PPI subnetwork. (D) Cluster-3 upregulated PPI subnetwork. Co-expression plots of (E) C3-MAPK10, (F) C3-MAPT and (G) MAPT-MAPK10 in cluster-3 AD (2515 cells) and control (862 cells) astrocytes respectively.

We propose that C3⁺ cells here might be corresponding to A1 neurotoxic reactive astrocytes. As discussed in detail above, GLUL, LDLR and TMED10 were found to be uniquely downregulated in cluster-3, and their downregulation was associated with increase in neurotoxicity during AD. For this reason, we investigated the interactions between the downregulated subnetwork genes in cluster-3 by highlighting these genes in the PPI network. As given below, these genes regulate different functions in astrocytes and we did not find any direct association between these genes in network results. Instead, we found association between these genes through TRIM E3 ubiquitin ligases (Figure 4.3C). TRIM E3 ubiquitin ligases, TRIM25 and TRIM67, were reported to have regulatory roles in brain development, behaviour (Boyer et al., 2018), AD pathology (Gómez-Tortosa et al., 2023) and neuroinflammation (Jia et al., 2022). Therefore, they might also be associated with the neurotoxic behaviours of A1 astrocytes. TRIM25 was found to be associated with both TMED10 and GLUL, and TRIM67 was found to be associated with TMED10 and LDLR.

In addition to the reported characteristics of A1 reactive astrocytes in 2017 (Liddelow et al., 2017), we also focused on their contribution to A β and NFT accumulation. Since MAPK10 expression was reported to cause an increase in A β and NFTs (Yarza et al., 2016) and found to be uniquely upregulated in cluster-3 AD astrocytes, we wanted to check the association of MAPK10 in C3⁺ astrocytes. For this purpose, we investigated the interactions between the upregulated subnetwork genes in cluster-3 in a PPI network by focusing on the association of C3 and MAPK10. Although we did not find direct association between C3 and MAPK10, we found that MAPK10 is associated with MAPT, which is the tau protein, and MAPT was also found to be upregulated in cluster-3 AD astrocytes (Figure 4D). We also found that MAPT was associated with important chaperone HSP90AA1, which is involved in AD pathology, and HSP90AA1 was found to be associated with C3. Moreover, we found that MAPK10 and MAPT are co-expressed in both cluster-3 AD and control astrocytes. Also, we observed co-expression between C3 – MAPK10 and C3 – MAPT. Overall, these results indicate that A1 astrocytes might have strong contribution to A β and NFT accumulation.

4.13. Alzheimer's Disease Leads to Epigenetic Alterations on Astrocyte Subtypes

Chromatin accessibility provides the occasion of the gene expression, and changes in the chromatin accessibility might lead to altered gene expression. As we added the chromatin accessibility component to the existent gene expression information, we were able to evaluate the astrocyte subtypes at multi-omic level and check whether they undergo epigenetic alteration or not. To this end, subtype specific differential promoter accessibility analysis was conducted by DESeq2. Differentially accessible promoter regions were identified for each cluster during AD. Then, by using the previously mentioned AD-related gene list compiled from the literature, AD related promoter regions were determined. Differentially accessible promoter regions, their corresponding genes and their numbers were given in Table 4.2.

Table 4.2: Alzheimer's Disease leads to epigenetic alterations on astrocytes. Increased and decreased terms correspond to increased and decreased chromatin accessibility.

Clusters	Increased	Decreased	AD-Related Increased	AD-Related Decreased
Cluster-0	78	48	2 (PSMA5, PAICS)	1 (SLC19A1)
Cluster-1	79	60	6 (NTRK1, TP63, IL12B, OLR1, ATG101, SERPINA3)	2 (DBH, BRSK2)
Cluster-2	72	41	4 (STAR, ATG2A, WNT11, NAE1)	1 (PPP3R1)
Cluster-3	68	85	4 (NDUFS5, TLR4, VDAC2, PSMC1)	2 (HRAS, TUBB4A)
Cluster-6	120	51	5 (SLC39A10, TUBB8, PSMD9, FZD2, MX1)	5 (PPP2R2A, NRBF2, CLUAP1, WNT9B, GGA3)
Subtype-unaware	145	124	7 (PSMA5, UBQLN1, CD44, SERPINA1, SERPINA3, TUBB3, TUBB6)	5 (UNC5C, LYN, DPYS, MAPK8IP1, SLC19A1)
Sum of unique genes in clusters	411	275	21	11

21 unique AD-related promoter regions were found to have increased accessibility and 11 unique AD-related promoter regions were found to have decreased accessibility during AD. Increased promoter accessibility of SERPINA3 and PSMA5 were also found to be increased in the overall AD phenotype, on the other hand decreased promoter accessibility of SLC19A1 was also found to be decreased in the overall AD

phenotype. Interestingly, AD-related differentially accessible regions identified from each cluster is unique.

4.14. Multi-omic Integrative Analyses Revealed Genes that Exhibit Alterations in Both Promoter Accessibility and Gene Expression

After identifying differentially accessible promoter regions in each astrocyte cluster, these findings were compared with differentially expressed genes identified in each cluster. We identified 29 genes that exhibit changes in both promoter accessibility and gene expression. Although promoter accessibility and gene expression were found to be positively correlated for certain genes, negative correlation was also observed. Table 4.3 shows certain genes that exhibit change in promoter accessibility and gene expression, and they were found to be strongly associated with astrocytic contribution to AD. Contrast between gene expression and chromatin accessibility may be caused by many different mechanisms, including post-transcriptional and translational processes. Here, due to existence of chromatin accessibility data, we focused on the TFs since they are capable of binding to promoter regions of genes of interests. Particularly, we focused on the TFs that are differentially up and downregulated in given clusters during AD. By this way, we narrowed down the possible targets that might regulate genes of our interest. In order to determine the possible regulators of the gene of interest, motif analysis was conducted. Motif analysis provides possible TF-gene interactions. In order to prove the exact binding of the TF, ChIP-seq data is required as it relies on the sequencing of the TF-bound DNA. For this reason, we supported our motif analysis results with ChIP-seq results taken from a comprehensive human transcription factor database, hTFtarget, (Q. Zhang et al., 2020), which contains thousands of human ChIP-seq datasets from various tissues.

GTPase Activating Rap/RanGAP Domain Like 3 (GARNL3) gene was found to be upregulated in dorsolateral prefrontal cortex (DLPFC), temporal cortex (TCX) and cerebellum (CBE) brain regions (from ROSMAP and Mayo Clinics brain bank studies) during AD (Bayraktar et al., 2021). GARNL3 gene was found to be downregulated in AD astrocytes in all clusters, except for cluster-3 and cluster-6, and overall AD astrocyte phenotype. In addition, its chromatin accessibility was found to be significantly reduced in cluster-0 AD astrocytes and overall AD astrocyte phenotype.

It was found to be associated with DDX39A in cluster-0 (downregulated subnetwork), which was previously found to be related with microglial activation and cognitive decline in ROSMAP AD cohort (Patrick et al., 2021). Cell specific regulation of GARNL3 during Alzheimer's disease have not yet been completely enlightened, but, as transcriptomic and epigenetic responses of astrocytes and some astrocyte subtypes are different from the bulk tissue transcriptomic studies for the similar tissue e.g. DLPFC, potential roles and regulators of GARNL3 in AD might be important for disease pathology. Moreover, our motif analysis results showed that ATF3, ZNF680, FOS and HES5 TFs might regulate the transcription of GARNL3 as they might bind to its promoter. ATF3 and FOS were previously reported to bind to GARNL3 promoter in the hTFtarget database, thus validating our results.

Table 4.3: The list of genes exhibiting changes in both promoter accessibility and gene expression in the same cluster during AD and possible transcriptional regulators of these genes in given clusters. “Upregulated TFs” and “Downregulated TFs” columns are representing the up and downregulated transcription factors in given clusters based on snRNA-seq data and these transcription factors were identified to be capable of binding to the promoter regions of the corresponding genes based onto our motif analysis results. Asterisk (*) refers to TFs that were previously reported to regulate corresponding gene based on the information taken from hTFtarget database.

	Genes	Gene Expression	Chromatin Accessibility	TFs that have Motifs in Gene Promoter	
				Upregulated TFs	Downregulated TFs
Cluster-0	GARNL3	Downregulated	Decreased	ATF3*, ZNF680, FOS*	HES5
	SLC1A2	Downregulated	Increased	ATF3*, BCL6, PBX3*, RFX2	SOX9
	GCLM	Upregulated	Decreased	ZSCAN31, PBX3*, NR4A1, FOS*, RFX2*	
	CHI3L1	Upregulated	Decreased	RARB, ZSCAN31, FOXK1, ZNF680, PBX3*, FOS*	
Cluster-1	PTN	Downregulated	Increased	ATF3*, FOS*, TGIF1	
Cluster-3	GLUL	Downregulated	Decreased	NR4A2, NR1D2, THRB, RARB, ZNF680, HMBX1, MXI1*, RFX2, ZNF331	JUN, NFIA, STAT1*, ETV1*, PKNOX2, HIF1A, FOS*, MEIS2, SOX9, JUNB
Cluster-6	SLC6A11	Downregulated	Decreased		
	SDC4	Downregulated	Increased		

As it was mentioned above, SLC1A2 was reported to regulate glutamate metabolism (Wood et al., 2022) and its downregulation might reduce neurotransmitter homeostasis

in CNS. SLC1A2 is mainly expressed by astrocytes in CNS (Wood et al., 2022) and A β was found to induce SLC1A2 mis-localization and internalization in astrocytes, and this leads to reduced glutamate uptake from synaptic cleft (Scimemi et al., 2013). A recent post-mortem human brain study proposed that high SLC1A2 expression in astrocytes during AD was neuroprotective (Kobayashi et al., 2018). SLC1A2 was found to be downregulated in AD astrocytes in all clusters and the overall AD astrocyte phenotype based on DGEA results. Thereby, SLC1A2 downregulation might indicate the loss of protective functions. Interestingly, the promoter accessibility of SLC1A2 was found to be increased in cluster-0 although reduced gene expression and increased chromatin accessibility might be referring to a neuroprotective nature for cluster-0. Due to increased chromatin accessibility, enhanced gene expression might be expected but instead gene expression was reduced. One possible mechanism that might be causing reduced gene expression is binding of repressor transcription factors to the SLC1A2 promoter. In our motif analyses, we found that, ATF3, BCL6, PBX3, RFX2 and SOX9 might regulate transcription of SLC1A2 as they can bind to its promoter. ATF3 and PBX3 were previously reported to regulate SLC1A2 in hTFtarget database.

Glutamate-Cysteine Ligase Modifier Subunit (GCLM) is involved in NRF2 system, which is a defence mechanism against mitochondrial oxidative stress. GCLM expression in astrocytes was found to be increased when they were exposed to tau (Ungerleider et al., 2021). NRF2 activation in astrocytes was previously reported to be protective against neurodegeneration in Amyotrophic Lateral Sclerosis (ALS) (Vargas et al., 2008). GCLM was found to be upregulated in cluster-0,1,2,3 AD astrocytes and the overall AD astrocyte phenotype. Thereby, it might be considered as a neuroprotective behaviour. On the other hand, chromatin accessibility of GCLM gene was found to be decreased in cluster-0 AD astrocytes. Due to the reduced chromatin accessibility, reduction in gene expression might be expected but gene expression was found to be increased. Here, some activator transcription factor might be regulating the abundance of GCLM mRNA in cluster-0. Our motif analyses showed that, ZSCAN31, PBX3, NR4A1, FOS and RFX2 can bind to GCLM promoter and possibly regulate its transcription.

CHI3L1 encodes for a protein called YKL-40 and this protein was reported to be CSF biomarker for AD (Lananna et al., 2020). It also has been reported to be associated with astrocyte reactivity and neuroinflammation (Dai et al., 2023). In APP/PS1 mouse

AD model, Chi3l1 depletion was shown to reduce amyloid plaque burden (Lananna et al., 2020). Its expression has been proposed to suppress phagocytic activation of glial cells and promote A β accumulation (Lananna et al., 2020). CHI3L1 was found to be upregulated in AD astrocytes in all clusters and overall AD astrocyte phenotype except for cluster-7. Therefore, the upregulation of CHI3L1 in astrocytes might be neurotoxic. On the other hand, its promoter accessibility was shown to be reduced significantly in cluster-0 AD astrocytes. Thereby, this altered epigenetic behaviour might be considered as neuroprotective, and possible transcriptional regulators of CHI3L1 in cluster-0 might be investigated. Our motif analyses showed that RARB, ZSCAN31, FOXK1, ZNF680, PBX3 and FOS TFs can bind to CHI3L1 promoter and might be its possible regulators.

PTN is a cytokine and expressed in human brain and its expression level was shown to be increased in response to inflammatory triggers that might be caused by neurodegenerative diseases (Fernández-Calle et al., 2017). In a recent study, it has been proposed that PTN might be an important regulator in neuroinflammation (Fernández-Calle et al., 2017). PTN was reported to be upregulated in AD (Skillbäck et al., 2017). However, in entorhinal cortex and superior frontal gyrus astrocytes, it was found to be downregulated (Leng et al., 2021). PTN upregulation in multiple sclerosis astrocytes was shown to be neuroprotective as PTN was shown to reduce proinflammatory signalling in astrocytes and promotes neuronal survival (Linnerbauer et al., 2022). In our case, PTN gene was found to be downregulated (adjusted p-value 1.34×10^{-40}) in cluster-1 AD astrocytes (Figure 4.4B). Downregulation of PTN might be considered to be a neurotoxic behaviour. On the other hand, its promoter accessibility was found to be significantly increased (p-value 0.00127) in cluster-1 AD astrocytes (Figure 4.4A).

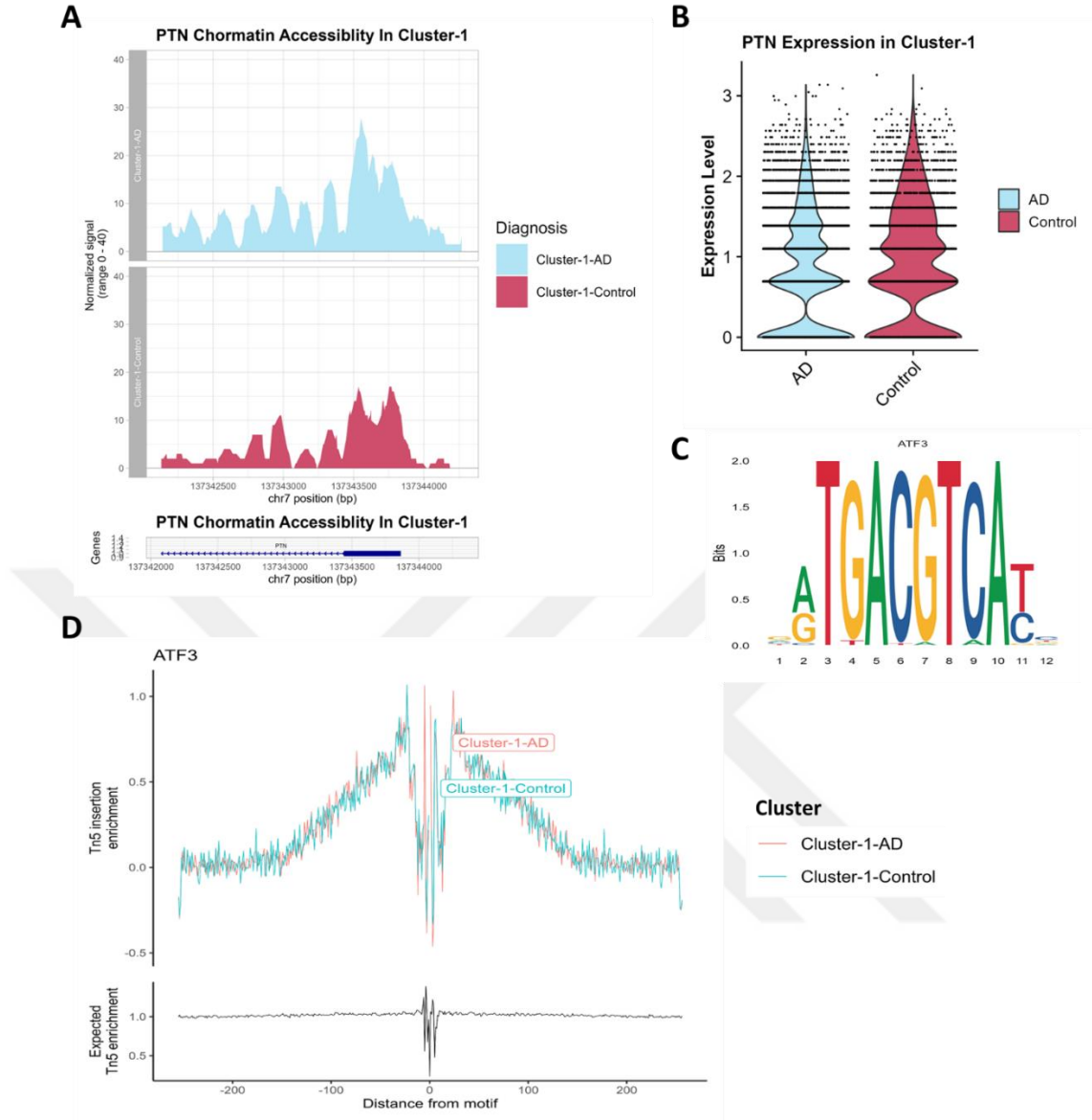


Figure 4.4: PTN might be regulated by ATF3 transcription factor. (A) Promoter accessibility of PTN gene in cluster-1. (B) PTN gene expression in cluster-1 in AD and control astrocytes. (C) ATF3 TF motif. (D) Motif foot-printing result shows that ATF3 binds to certain genes in cluster-1.

Increased promoter accessibility of PTN in cluster-1 might be associated with the regulation of PTN in cluster-1 by TFs. We searched for possible transcription factors that might regulate PTN expression. We found that ATF3, FOS and TGIF transcription factors are upregulated in cluster-1 during AD based on our snRNA-seq data. Moreover, motif analysis was conducted, and it was found that ATF3 and FOS transcription factors are able to bind to PTN promoter.

Accuracy of the motif analysis results were supported with hTFtarget database as they also reported that ATF3 can bind to PTN promoter. Also, motif foot-printing analysis was performed for ATF3 TF, and it was validated that it binds to certain gene promoters in cluster-1 (Figure 4.4C, D). As it can be seen from the Figure 4.4D, Tn5 insertion events is increasing up to some point, then starts to decrease as it approaches to the centre of the motif due to TF existence in the promoter region. Eventually, PTN expression was obtained to be downregulated in cluster-1 AD astrocytes and this situation might be considered as neurotoxic. Increased chromatin accessibility of PTN in cluster-1 might be referring to neuroprotective behaviour in epigenetic level. But this behaviour does not seem to appear in mRNA level.

Cell surface heparan sulfate proteoglycans (HSPGs) have important contributions to AD pathology as they regulate cellular uptake of A β , and they mediate its attachment and internalization into the cells (Letoha et al., 2019). Syndecan-4 (SDC4) and Syndecan-3 (SDC3) are a family of HSPG, and they were reported to co-localize with amyloid plaques and their expression were reported to increase in postmortem AD brains (Letoha et al., 2019). SDC4 and SDC3 were also shown to trigger fibrillation of A β (Letoha et al., 2019). SDC4 was reported to be the astrocyte-specific syndecan (Lian & Zheng, 2016b). It was proposed that SDC3 and SDC4 might be an important target for A β to enter inside the cells, and overexpression of SDC3 and SDC4 can contribute to the uptake and fibrillation of A β (Letoha et al., 2019). We found that SDC3 was uniquely downregulated in overall AD astrocyte phenotype. On the other hand, SDC4 was found to be downregulated in AD astrocytes in all clusters and overall AD astrocyte phenotype. Thereby, this mechanism might be leading to reduced uptake of A β . But, strangely, chromatin accessibility of SDC4 was found to be increased in cluster-6 AD astrocytes. SDC4 mediated internalization of A β by astrocytes might be investigated for potential therapeutic roles for AD.

Chromatin accessibility of GLUL and SLC6A11 genes were reduced in cluster-3 and cluster-6 AD astrocytes. Reduced chromatin accessibility might be causing reduction in gene expression in these clusters and these might be considered as a neurotoxic behaviour due to the reduced neurotransmitter homeostasis. Motif analyses revealed many possible regulators of GLUL genes, including NR4A2, NR1D2, THRB, RARB, ZNF680, HMBOX1, MXI1, RFX2, ZNF331, JUN, NFIA, STAT1, ETV1, PKNOX2,

HIF1A, FOS, MEIS2, SOX9 and JUNB. MXI1, STAT1 and ETV1, which were previously reported to regulate GLUL expression in hTFtarget database.

4.15. Detection of Astrocyte-Subtype Specific Genes in Comprehensive Bulk Transcriptome Dataset Through Deconvolution

An additional analysis was performed to check if astrocyte-specific transcriptomic signatures obtained from the subtype-specific analysis could also be identified from bulk transcriptome data through deconvolution. Since astrocyte specific comprehensive bulk transcriptome datasets are not available for AD, commonly used AD bulk RNA-Seq datasets in the literature were processed by using the deconvolution approach. To this end, we used the bMIND deconvolution algorithm (J. Wang et al., 2021) to predict sample-level cell-type specific gene expression from bulk transcriptome data. bMIND first predicts cell type compositions from a bulk dataset by using single-cell based count matrix. Then, it uses this information to predict cell-type specific gene expression. As we analysed and annotated the Sadick dataset, we used the count matrix of this dataset as the input for the bMIND algorithm. Firstly, both bulk and single cell count matrices were normalized with Transcripts per Million (TPM) normalization method. Then, the count matrix of the Sadick dataset was used to calculate prior cell-type-specific profile and covariance matrix by using `MIND::get_prior` function. Then, this information was used to predict the cell-type fractions in each bulk sample within four major bulk transcriptome datasets for AD; ROSMAP (n:569, 404 AD and 165 control, syn17024112), Mayo Clinic (n:160, 82 AD and 78 control, syn9738945), Mount Sinai Brain Bank (n:184, 158 AD and 26 control, syn7416949) and GSE125583 (n:287, 217 AD and 70 control) datasets. Overall, the four datasets cover about 1000 AD patients. Cell type fractions were identified (Table 4.4) for each patient and control sample in those datasets through non-negative least squares (NNLS) approach by using `MIND::est_frac`. Mean predicted cell-type fractions for all samples for astrocytes, oligodendrocytes, microglia and neurons are given in Table 4.4 for each dataset. Then, to predict cell-type-specific expression profiles for each sample, covariance matrix and cell-type fractions were used as an input to `MIND::bMIND` function. At the end, sample level astrocyte-, oligodendrocyte-, microglia- and neuron- specific gene expressions were identified for each sample in each dataset.

bMIND also allows for differential testing and it was implemented to identify differentially expressed genes in deconvolved astrocytes during AD. Number of differentially expressed genes in astrocytes during AD were also given in Table 4.4. The highest number of differentially expressed genes were identified in the Mayo Clinic's dataset (2628 genes, Benjamini-Hochberg adjusted p-value < 0.001) and the lowest number of differentially expressed genes were identified in the MSBB dataset (142 genes, p-value < 0.05) study.

Table 4.4: Deconvolution results of the comprehensive AD transcriptome datasets. P-value 0.05 cut-off was implemented for DEGs in the ROSMAP, MSBB-PHG and GSE125583 datasets while adjusted p-value cut-off of 0.001 was used for the Mayo Clinics dataset. (DEGs: Differentially expressed genes; SUC: Subtype-unaware comparison; MSBB-PHG: Mount-Sinai Brain Bank - Parahippocampal Gyrus)

Average Cell-type Fractions (%)	ROSMAP	Mayo Clinics	MSBB PHG	GSE125583
Astrocytes	0.23	0.18	0.11	0.23
Oligodendrocytes	0.07	0.19	0.13	0.17
Microglia	0.14	0.18	0.17	0.24
Neurons	0.56	0.46	0.58	0.36
DEGs in AD Astrocytes	ROSMAP	Mayo Clinics	MSBB PHG	GSE125583
Number of DEGs	202	2628	142	779
Number of AD-Related DEGs	4	129	7	18
Number of Common DEGs with SUC	15	139	12	51

AD-related differentially expressed genes were also identified as it was reported in previous sections. The highest number of AD-related genes were identified in the Mayo Clinic's dataset. Moreover, differentially expressed genes identified by bMIND were also compared with the genes that were identified by subtype-unaware comparison earlier. 139 genes were found to be intersecting between subtype-unaware comparison and bMIND differential testing results for the Mayo Clinic's dataset.

Lastly, we investigated the expressions of astrocyte-specific genes that were either associated with neuroprotectivity, neurotoxicity or altered gene expression or chromatin accessibility, identified in the previous sections (C3, MAPK10, MAPT, LDLR, GLUL, TMED10, GARNL3, SLC1A2, GCLM, CHI3L1, PTN, SLC6A11, SDC4, ATF3, PLCB1, S100A6, ITM2B, ITM2C, SOD2, NQO1, AQP4). These genes were not found to be differentially expressed in astrocytes during AD in ROSMAP,

MSBB-PHG and GSE125583 based on the predicted astrocyte-specific gene expression profiles. However, MAPK10, MAPT, TMED10, AQP4 and ATF3 were found to be differentially expressed in astrocytes in Mayo Clinic's dataset.

As a summary, it is possible to obtain some of the subtype-specific differentially expressed genes from bulk transcriptome data by deconvolution. Nevertheless, we were unable to capture all the differentially expressed disease-related genes that were identified from the single-nuclei data. The results also show that the deconvolution efficiency of the algorithm, bMIND, varies between datasets significantly in terms of capturing AD-related genes in astrocytes. Therefore, single-nuclei or single-cell level omic data combined with subtype-specific analysis is crucial to capture genes that have a role in astrocyte-based response to AD.

5. CONCLUSIONS

AD is a neurodegenerative disorder that leads to cognitive impairment. Genetic factors, type 2 diabetes, obesity, traumatic brain injuries, stress, age, infections, smoking, air pollution are potential risk factors for AD. Despite the fact that AD was considered to be a neuron-based disorder, recent studies have shown that the contribution of glial cells to AD pathology is undeniable. Due to the conflicting behaviours of astrocytes during AD, their behaviours requires in-depth analysis. For this purpose, astrocytes obtained from comprehensive single-nuclei transcriptome and epigenome datasets for AD, which were generated from post-mortem human brain prefrontal cortex, (Morabito et al., 2021; Sadick et al., 2022), were re-analysed in this thesis study to identify astrocyte-subtype-specific transcriptomic and epigenetic responses to AD.

Our single-nuclei data integration and network-based analysis methods were proven to capture disease related genes with a higher sensitivity compared to the Sadick study (Sadick et al., 2022). The subnetwork genes discovered by KPM for each condition in each subtype were subjected to gene ontology analyses independently, and altered biological processes were identified in each subtype. The results showed that astrocyte subtypes exhibited heterogeneity during AD. Apart from gene ontology analysis, the subnetwork genes were also used for the identification of subtype specific neuroprotective and neurotoxic behaviours. Cluster-2 AD astrocytes mainly exhibited neuroprotective behaviour due to *PLCB1*, *S100A6*, *ITM2B*, *ITM2C*, *SOD2*, *NQO1* and *AQP4* expressions. On the other hand, cluster-3 AD astrocytes probably represent human A1 reactive astrocytes as they differentially express C3.

Some uniquely expressed transcripts were also identified in cluster-3 AD astrocytes that might be associated with NFT accumulation. *MAPK10* was previously reported to increase A β and NFT formation, and, in the results obtained in this thesis study, it was found to be uniquely upregulated in cluster-3 AD astrocytes. Due to its upregulation in cluster-3 AD astrocytes, its association with A1 reactive astrocyte phenotype was also investigated. Although there was not any direct association between C3 and *MAPK10*; they were found to be co-expressed by AD and control astrocytes in cluster-3. Since they were co-expressed in cluster-3, *MAPK10* might be associated with neurotoxic characteristics of A1 reactive astrocytes. The results also

showed that MAPK10 was associated with MAPT, and MAPT was also upregulated in cluster-3 AD astrocytes. MAPT is mainly expressed by neurons, but it was also shown to be expressed by astrocytes. It is known that A β binds to calcium sensing receptors in human astrocytes, and this leads to production and release of phosphorylated tau by astrocytes. Therefore, astrocytes can also contribute to NFT accumulation. Although no direct association was identified between MAPT and C3 in this thesis study, MAPT and C3 were found to be co-expressed in cluster-3 AD astrocytes. Their co-expression in cluster-3 AD astrocytes might indicate the role of A1 neurotoxic astrocytes to enhance the production of NFTs, worsening the disease pathology. Overall, these results indicate that A1 astrocytes might have strong contribution to NFT formation.

Moreover, snATAC-seq data for the same tissue was analysed by transferring the labels of snRNA-seq astrocytes to snATAC-seq astrocytes, and the majority of RNA-seq based clusters were identified in snATAC-seq astrocytes. Cluster-specific analysis of differentially accessible chromatin identified genes that exhibited change in promoter accessibility during AD. As a result of cluster-specific analyses, genes exhibiting both altered promoter accessibility and gene expression during AD were identified. Through focusing on these genes, PTN gene in cluster-1 was predicted to be regulated by ATF3 transcription factor. PTN and ATF3 TF interaction was previously shown through ChIP-seq, validating the results of this thesis study. The downregulation of PTN gene was previously reported to cause the loss of astrocytic neuroprotective functions, and, as it was found to be downregulated in cluster-1, this behaviour might be corresponding to the same phenomenon.

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PUBLICATIONS AND PRESENTATIONS FROM THE THESIS

A. İlğün, T. Çakır, (2023), “Functional Specificity Of Astrocyte Subtypes In Alzheimer’s Disease: A Multi-Omic Approach By Mapping Single Nuclei Data On Protein Interaction Networks”, International Symposium on Health Informatics and Bioinformatics

A. İlğün, T. Çakır, (2022), “Network-based approaches reveal cell subtype specific roles of astrocytes and oligodendrocytes during Alzheimer’s disease”, International Symposium on Health Informatics and Bioinformatics



APPENDICES

APPENDIX-A: QUALITY CONTROL METRICS FOR SADICK AND MORABITO DATASETS (CD)

APPENDIX-B: DETAILED ANALYSIS AND ANNOTATION STEPS FOR SADICK AND MORABITO DATASETS (CD)

APPENDIX-C: DIFFERENTIAL TESTING RESULTS FOR DATASET ANNOTATIONS AND INTEGRATED ASTROCYTES (CD)

APPENDIX-D: DIFFERENTIAL GENE EXPRESSION ANALYSIS AND NETWORK-BASED RESULTS FOR INTEGRATED ASTROCYTES (CD)