

# **Comparison of Major Marker Genes in Post-Mortem Alzheimers Disease Patients to Control Brain Samples**

CPSC 545 Final Project

Iman Mir

University of British Columbia

## **Abstract**

Alzheimer's disease (AD) is a neurodegenerative disease which affects cognitive capabilities in patients [1]. The study investigates major marker genes in samples from the NCBI Gene Expression Omnibus through single cell RNA sequencing (scRNA-seq) methods of Seurat and SingleR analysis. Post-mortem brain samples from two patients with AD and two control brain samples were investigated. It was determined that AD samples exhibited major genes of ADAM28, ZNF148, FLT1, CSF3R, LPAR5, CTNNA3, and SLC1A2. The control samples had CUX2, NORAD, RFN219-AS1, GRB14, SEMA3E and VCAN. Thus, the study determined the differentiation in the major gene clusters and developed a greater understanding of the underlying mechanisms associated with AD.

# Introduction

AD is a form of dementia which primarily effects patients at ages under 65 (early onset) or over (late onset) [1]. This condition is classified as a neurodegenerative disease which causes impairments in cognition and behaviour, including judgement, memory, language and comprehension [11]. Many common symptoms of the disease is episodic short term memory loss as well as sparing of long term memory. To add, further neuropsychiatric symptoms such as agitation, withdrawal, psychosis, apathy and withdrawal can occur in patients during late stages of the disease as well. Moreover, the development of brain lesions is the main cause of the pathological process of the disease. These lesions are hyperphosphorylated tau proteins and beta amyloid peptides which create neurotic plaques (NPs), neuropil threads (NTs) and neurofibril tangles (NFTs) within cell bodies [2]. The formation of these lesions cause cells to dysfunction and ultimately leads to AD.

Furthermore, the progression of AD can be categorized based on the Braak staging system which describes the pathology of NFTs in the medial temporal lobe of patients [13]. In Braak stages one and two, there are low concentrations of NFTs in the entorhinal cortex and transentorhinal cortex where individuals typically experience normal cognitive function. In Braak stages three and four, NFTs accumulate in the neocortex and hippocampus regions of the brain and cognitive dysfunction can begin to appear in patients. Finally, in Braak stages four and five, the highest concentration of NFTs are found throughout brain regions and is associated with the highest level of cognitive impairment [13].

To add, computationally determining cellular types can be completed by implementing scRNA-seq methods [3]. High-throughput single-cell transcriptomes datasets can be used in order to determine tissue models and cell types [3]. Clustering techniques can also be applied in order to organize various genes into clusters with similar functions from these datasets [14]. Therefore, in order to investigate the major types of genes in human AD patients, this paper will investigate the post-mortem brain samples from two patients with AD from Braak stages three and four. These samples will be compared to control brain samples to determine the differentiation in the major gene clusters and their functionalities. The dataset contains gene barcodes, features and matrices and is analyzed through the Seurat R toolkit [3]. The toolkit consists of packages which aims to allow users to interpret heterogeneity from single-cell transcriptomes and integrate data as well [7]. Thus, these methods will aid in the identification of the gene clusters within the samples and the determination of major gene types.

## Methods

ScRNA-seq data was obtained from the NCBI Gene Expression Omnibus and 4 samples were analyzed [22]. Two samples were from human AD brain regions in Braak stages three/four and two control brain regions were analyzed. Overall, the determination of the marker genes for the AD samples and control brain samples were completed in three stages, the first stage was the Seurat pre-processing workflow ([https://satijalab.org/seurat/articles/pbmc3k\\_tutorial.html](https://satijalab.org/seurat/articles/pbmc3k_tutorial.html)), the second stage was the Seurat cluster analysis and the third stage was the SingleR cluster analysis. In the preprocessing workflow, all four sample directories were read into R studio through the Read10X function and four Seurat objects were created. Thereafter the AD objects and control objects were combined together in order to ensure higher specificity in determining marker gene and identifying common genes that are prevalent in both samples. Thereafter PercentageFeatureSet was conducted on the two datasets in order to calculate the percentage of the counts which belong to mitochondrial genes. In general, cells that are often of low quality with few genes or that are dying can have high rates of mitochondrial contamination and therefore these types of cells are removed from the data. The QC metricises are determined for each sample (Figure 1 and Figure 2) and the data is subsetted and normalized to remove the low quality cells.

Thereafter, highly variable features were determined (see Appendix Figure 1) for each sample which highlights features that exhibit high cell to cell variations. The data was also scaled to ensure certain highly expressed genes within the dataset did not dominate the clusters and the mean expression of the cells are 0 and the variance is 1. Linear dimensional reduction (PCA) was also completed on the data based on the variable features determined (see Appendix Figure 7). After, the cells were clustered based on the FindNeighbours and FindClusters functions in order to determine the different clusters in each sample and UMAPs were created as well to visualize the data.

After the pre-processing workflow was completed, the second stage of cluster analysis with Seurat was conducted. Through the FindAllMakers function, all the marker genes for the dataset for the AD and controls were found. Thereafter the top genes per cluster was determined by creating a function which mapped the clusters and determined the top gene. Based on the data, feature plots (Figure 3) of the top marker genes were visualized in order to determine if the marker genes were localized into a distinct cluster. After all marker genes were compared, a second UMAP was completed (Figure 4) with the different genes. Thereafter, in order to have a wider range of data comparisons, the third stage of SingleR analysis was conducted. The reference HumanPrimaryCellAtlasData was used to compare each cluster with different cell types. Rather than genes, the type of cells found within the clusters were determined.

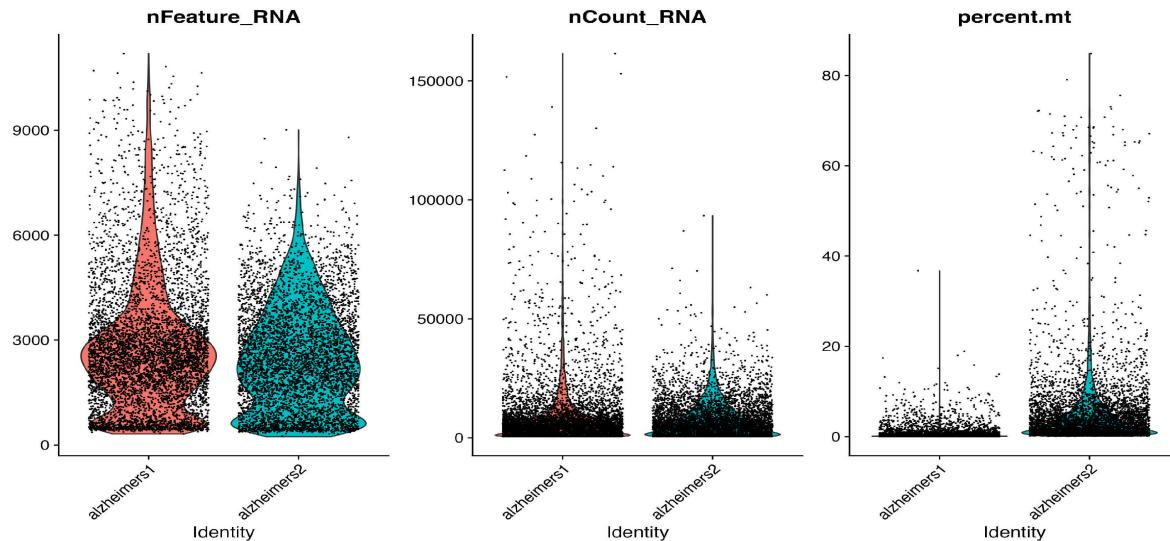
The code for the analysis methods can be found on this public repository: <https://github.com/imam-mir/Alzheimers-Disease-Analysis>

# Results

## Pre - Processing Data Analysis

The following figures outline the pre-processing methods completed on the AD and controls combined datasets.

A



B

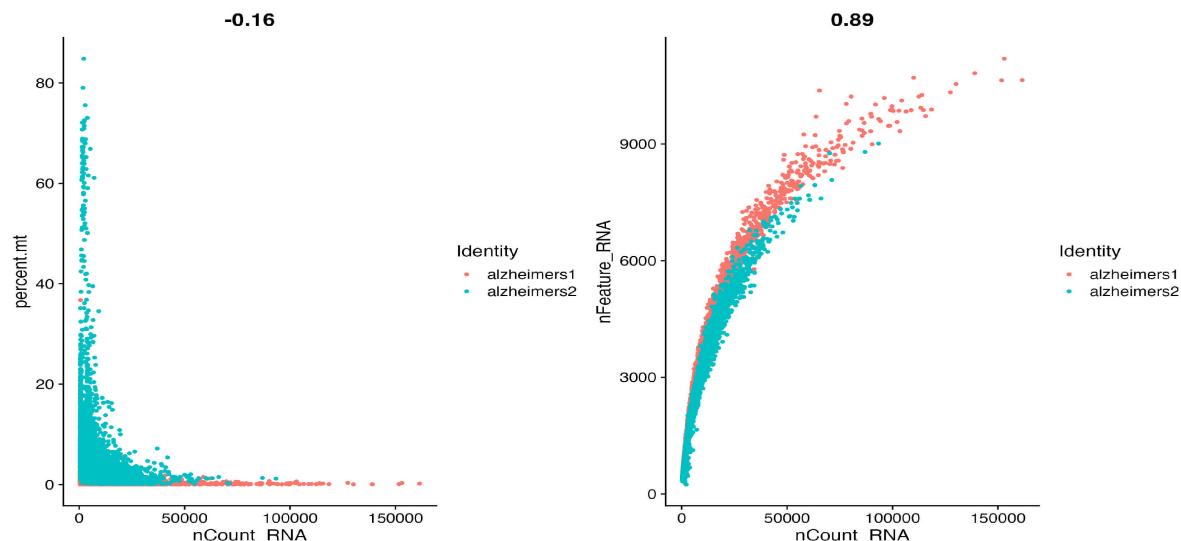


Figure 1: AD Combined Data QC Metricises

The QC metricises for the AD samples were visualized through a violin plot (A) outlining the nFeature\_RNA, nCount\_RNA and percent.mt for the data. The feature scatter plots (B) also determined the percent.mt vs nCount\_RNA and nFeature\_RNA vs nCount\_RNA for the data.

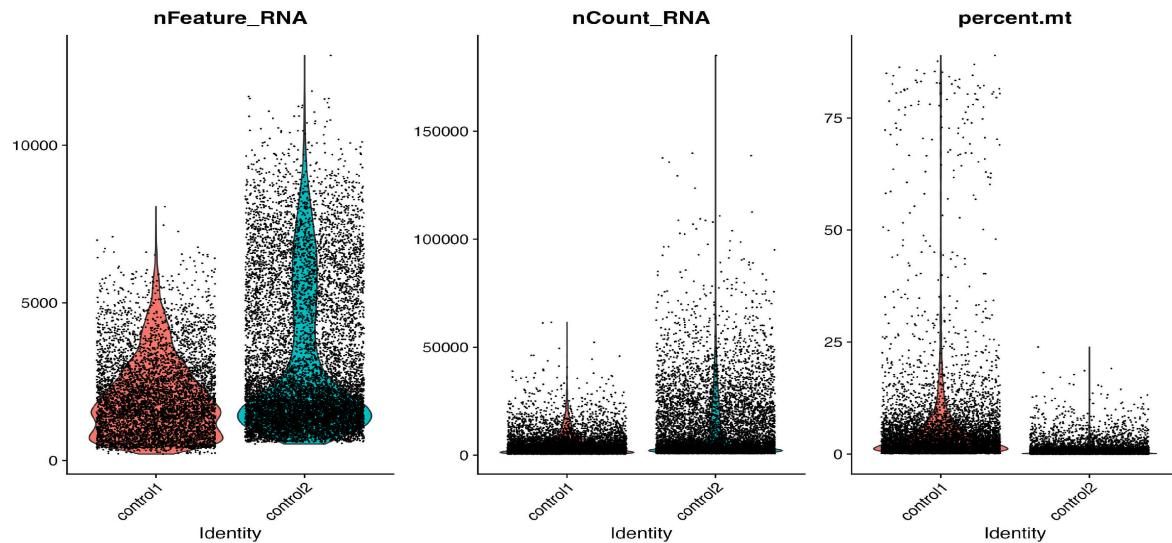
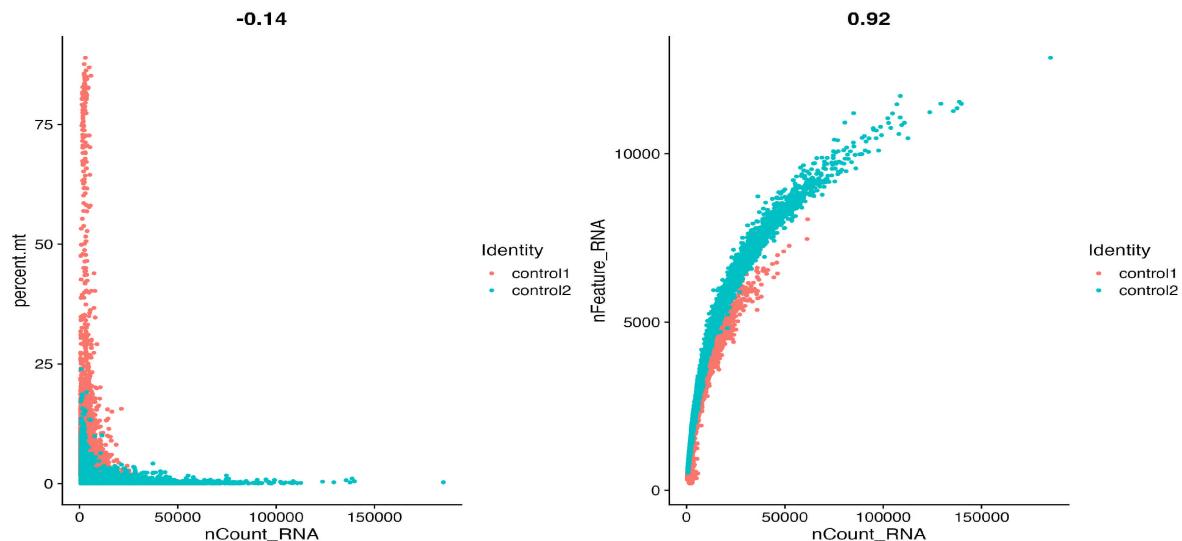
**A****B**

Figure 2: Control Combined Data QC Metricises

The QC metricises for the control samples were visualized through a violin plot (A) outlining the nFeature\_RNA, nCount\_RNA and percent.mt. Feature scatter plots (B) were also determined, representing the percent.mt vs nCount\_RNA and nFeature\_RNA vs nCount\_RNA

## Seurat Cluster Analysis

All major genes for the samples were determined through the Seurat R package and the top markers for each cluster were identified.

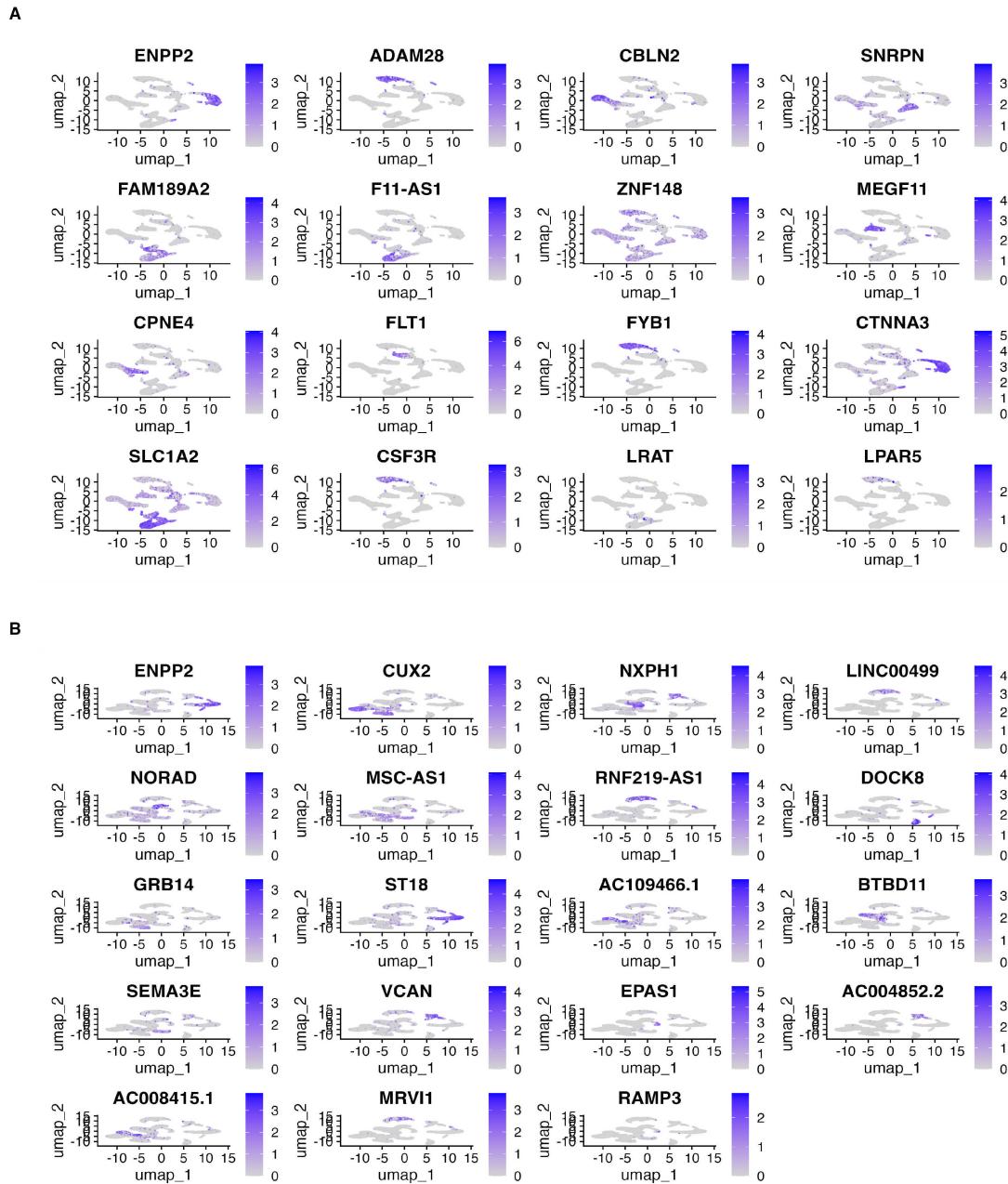


Figure 3: Feature Selection of Samples

A total of 16 clusters were identified for the AD samples (A) and a total of 19 clusters were identified for the control samples (B).

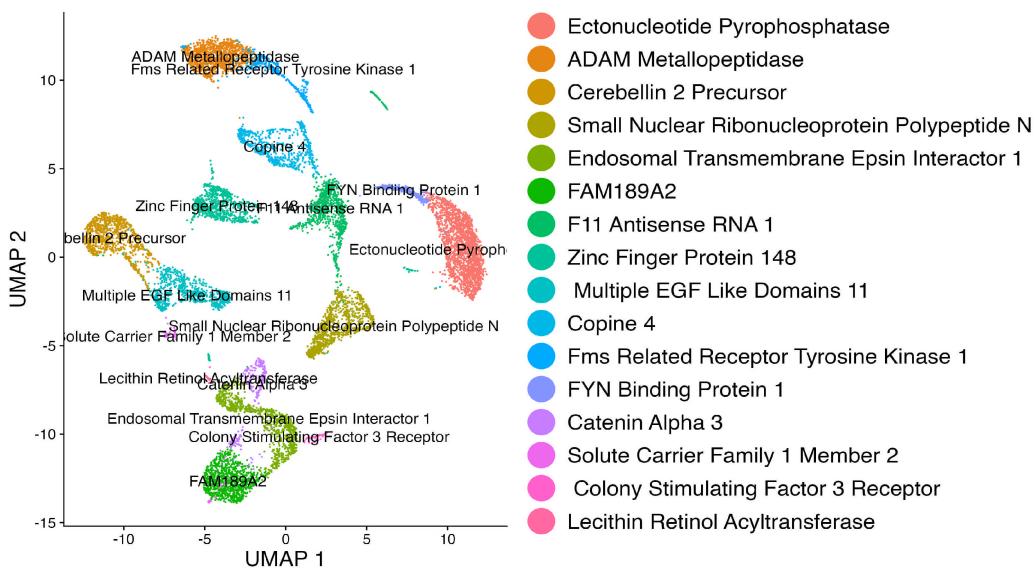
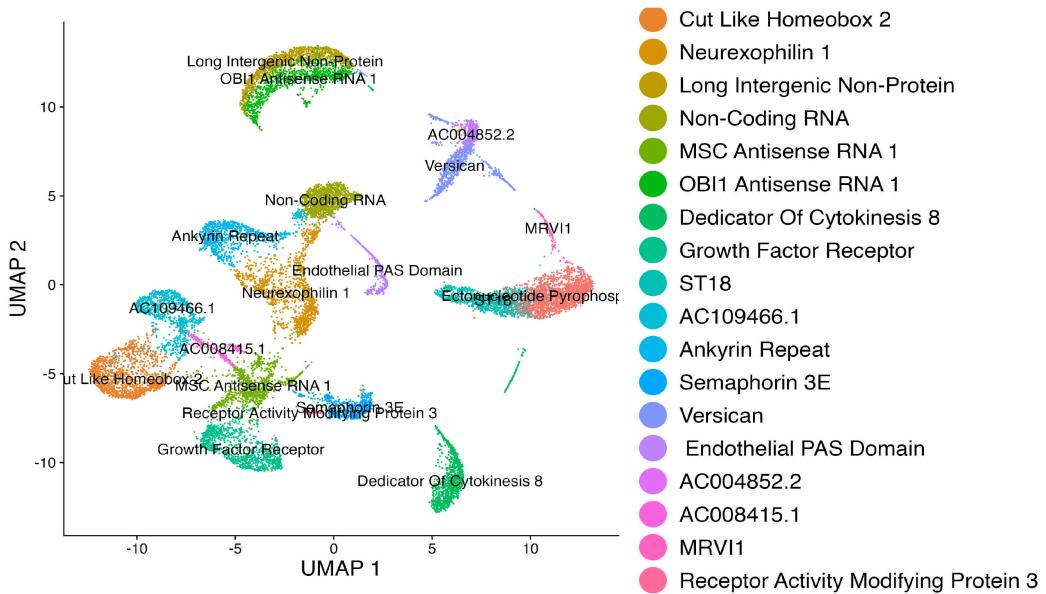
**A****B**

Figure 4: UMAP Plots of Samples

Based on the feature selection of the samples, the major marker genes for the clusters were identified. Several different proteins, receptors and other structures were highlighted for the AD data (A) and control data (B).

## SingleR Cluster Analysis

The major cells were determined through the SingleR package and the cluster data was compared to the Human Primary Cell Atlas Data for reference.

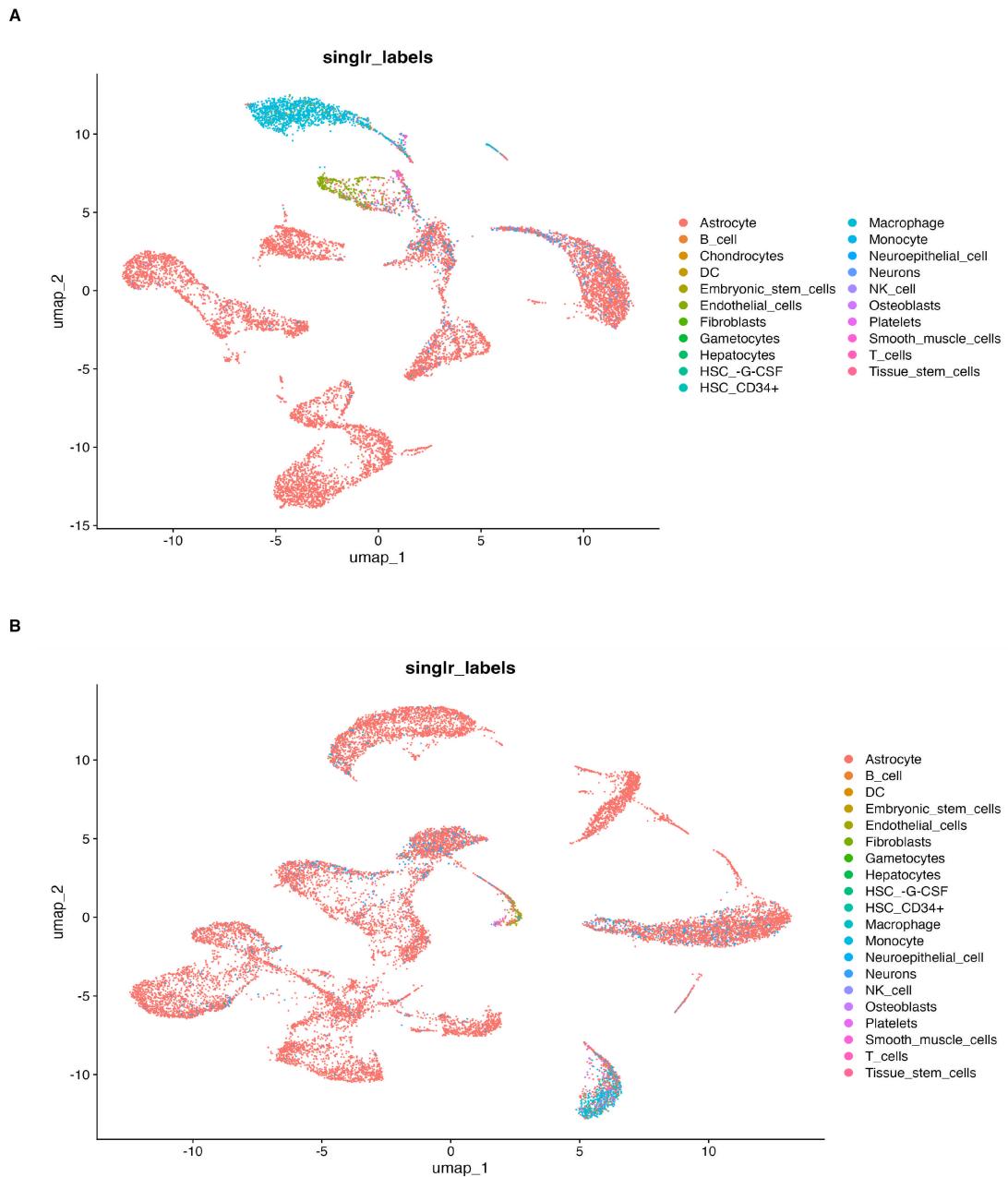


Figure 5: SingleR UMAP Plots

Based on the comparisons between the clusters to the reference data in the AD data (A) and control data (B), the R package determined different cell types in each cluster.

# Discussion

## AD Pathology

In general, various transcriptionally regulatory networks and genes are impacted as a result of the progression of AD. For early onset AD, the main genes that are involved include presenilin 1, presenilin 2 and amyloid precursor protein [1]. These three genes increase amyloid beta protein production in cells leading to AD. Presenilin 1 and 2 are transmembrane proteins which are critical in gamma secretase activity. Amyloid precursor proteins genes in chromosome 21 encode for amyloid beta proteins, and increased mutations/triplication of the chromosome can result in an overexpression of the gene leading to AD pathology. Further, in late onset AD, the genes for apolipoprotein E are major genetic risk factors for the disease [1]. The gene is a cholesterol carrier in the brain and is part of brain neuronal signalling, glucose metabolism, lipid delivery and neuronal maintenance. A variation of the gene known as E4 is also found in many patients and is associated with the change in structure of the apolipoprotein E protein. To add, other genetic factors and non-genetic factors can cause altered amyloid beta protein production, aggregation, clearance, and inflammation leading to AD [1].

## Seurat Cluster Analysis

Based on Figure 3A, the main marker genes for the AD samples were ENPP2, ADAM28, CBLN2, SNRPN, FAM189A2, F11-AS1, ZNF148, MEGF11, CPNE4, FLT1, FYB1, CTNNA3, SLC1A2, F11-AS1, CSF3R, LRAT and LPAR5. The marker genes for the control samples (Figure 3B) were ENPP2, CUX2, NXPH1, LINC00499, NORAD, MSC-AS1, RNF219-AS1, DOCK8, GRB14, ST18, AC109466.1, BTBD11, SEMA3E, VCAN, EPAS1, AC004852.2, AC008415.1, VCAN, MRVI1, DOCK8 and RAMP3. Further, the names of these marker genes were determined through the NCBI gene sources directory in order to create the labels for Figure 4. Based on NCBI, the genes in the AD samples translated to Ectonucleotide Pyrophosphatase, ADAM Metallopeptidase, Cerebellin 2 Precursor, Small Nuclear Ribonucleoprotein Polypeptide N, Endosomal Transmembrane Epsin Interactor 1, FAM189A2, F11 Antisense RNA 1, Zinc Finger Protein 148, Multiple EGF Like Domains 11, Copine 4, Fms Related Receptor Tyrosine Kinase 1, FYN Binding Protein 1, Catenin Alpha 3, Solute Carrier Family 1 Member 2, F11 Antisense RNA 1, Colony Stimulating Factor 3 Receptor, Lecithin Retinol Acyltransferase and Lysophosphatidic Acid Receptor 5. The genes in the control samples translated to Ectonucleotide Pyrophosphatase, Cut Like Homeobox 2, Neurexophilin 1, Long Intergenic Non-Protein, Non-Coding RNA, MSC Antisense RNA 1, OBI1 Antisense RNA 1, Dediator Of Cytokinesis 8, Growth Factor Receptor, ST18, AC109466.1, Ankyrin Repeat, Semaphorin 3E, Versican, Endothelial PAS Domain, AC004852.2, AC008415.1, MRVI1 and Receptor Activity Modifying Protein 3.

Both samples only exhibited two of the same genes which were ENPP2 and DOCK8. Specifically, ENPP2 (also known as Autotaxin), is an enzyme involved in creating lysophosphatidic acid and stimulating signalling pathways. Certain changes in the structure and expression of the gene is associated with cancer, inflammation, neural defects, and AD [18]. Further, DOCK8 is a gene primarily expressed in immune cells and is associated with neurological diseases like AD. The expression of DOCK8 increases beta amyloid peptide production and can lead to apoptosis of neuronal cells due to inflammation [26]. As a result, these genes both have regulatory roles in signalling pathways and immune responses in the control brain samples and in patients with AD,

overexpression of the genes can lead to inflammation and disease.

Further, there were multiple genes that were expressed in AD brain samples and not highlighted in the control brain samples. The biologically relevant genes of ADAM28, ZNF148, FLT1, CSF3R, LPAR5, CTNNA3, and SLC1A2 will be investigated for their roles in AD progression. ADAM28 is a membrane-anchored glycoprotein part of the neurogenesis process in the brain [9]. The gene is catalytically active and expressed in lymphocytes and is known to be involved in AD progression. Next, the gene ZNF148 is a zinc-finger protein involved in ubiquitin-mediated protein degradation, cell migration, transcriptional regulation, actin targeting and other processes [4]. ZNF148 has oncogene functions and is part of apoptosis, cancer metastasis and regulates the beta catenin pathway. Since the beta catenin signalling components are part of amyloid precursor protein processing, this suggests that ZNF148 may have a role in AD progression with altering amyloid beta production [1]. Further, FLT1 is involved in signalling and mediating chemotactic responses for immune responding cells [21]. In AD, the gene is involved in inflammatory responses and mobilizing microglial cells to peptide deposits such as amyloid beta proteins. The gene is also upregulated in AD and leads to further neurodegeneration within the brain.

In addition, the CSF3R gene is the receptor for the CSF3 protein involved in neutrophil functionality [25]. The gene is part of AD inflammatory processes and this type of inflammation within the peripheral blood of patients can lead to osteoporosis as well. Next, LPAR5 is a gene part of brain development and stimulated following nerve injuries [19]. The gene affects microglial structures through pro-inflammatory responses leading to neurodegenerative and neuropsychiatric disorders. CTNNA3 in late onset AD encodes for alpha-T catenin and is a binding partner for beta catenin [16]. It produces amyloid peptides which is a precursor for NPs, NTs and NFTs in cell bodies, causing AD [2]. SLC1A2 is also known as glutamate transporter-1 (GLT-1) which controls concentrations of the glutamate neurotransmitter in neurons and astrocytes [27]. In AD, the upregulation of glutamate transporters results in the formation of amyloid plaques and cognitive decline in patients citezoltowska2018novel. The transporter also interacts with presenilin 1's structural cytosolic loops which further causes mutations to the proteins leading to AD. Overall, these genes provide further understanding of AD progression and the subsequent decline in cognitive function in patients. Although these genes do not explicitly encode for the main proteins involved in AD as discussed before, they still have major roles in the progression of AD through inflammatory responses, signalling mechanisms, cancer metastasis and other roles.

The following major genes CUX2, NORAD, RNF219-AS1, GRB14, SEMA3E and VCAN are in the control brain samples which were not found in AD samples. These genes have significant roles in maintaining normal processes and functions in the brain. The transcription factor CUX2 is part of synapse formation, spine and dendrite development [15]. It is involved in corticogenesis and the development of adult cortical layers, which is essential for neocortical development in the brain. NORAD is a greatly conserved long non-coding RNA molecule which is upregulated after DNA damage and provides chromosomal stability [24]. It also participates in mitosis and ensures proper mitotic division of cells. RNF219-AS1 interacts with the CCR4-NOT complex as an acetylation-regulation cofactor. Specifically, it functions to inhibit the CCR4-NOT and controls mRNA turnover and degradation [20].

GRB14 functions to regulate the action of insulin and insulin receptors [8]. Specifically, after insulin binds to insulin receptors, autophosphorylation of tyrosine residues occurs and initiates the

signal cascade. GRB14 inhibits the catalytic activity of the receptor, blocks access to the substrates, recruits negative regulators and regulates protein tyrosine phosphatase access. SEMA3E in the brain interacts with neuropiles and binds to PlexinD1 to initiate signalling pathways which are crucial in the development of descending axon tracts [5]. These pathways function to establish neural circuits in the forebrain. VCAN is part of the extracellular matrix in the brain and has a crucial role in the growth of neurons and creating neural networks [23]. Within the protein, the G3 domain functions to enhance excitatory synaptic activity neurite growth and cell attachment. Therefore, the genes described have major roles within the brain and can allow for typical brain functioning.

The main differences between the major marker genes of the samples are their roles in maintaining brain functions. In AD samples, genes such as CTNNA3, ZNF148, CSF3 and FLT1 have roles in amyloid beta production, inflammatory responses and signal cascades. These roles are quite different in comparison to control samples where genes such as GRB14, SEMA3E, NORAD and VCAN have roles in maintaining proper cell and neural function. Thus, through the Seurat cluster analysis, the determination of the major marker genes was successful in analyzing genes that have major roles in the respective AD and control brain samples.

Although the genes described are accurate representations of the samples, it is possible that certain genes may have been removed during the subsetting of the data. As shown in Figure 1A, the nFeature\_RNA, nCount\_RNA and percent.mt between the two samples of AD are quite different. The biggest difference in the graphs is the percent.mt which is much higher in the alzheimers2 data in comparison to the alzheimers1 data. Comparatively in Figure 1B, the control1 data has a higher percent.mt in comparison to the control2 data. As a result, in order to properly subset the data, upper bound and lower bound values were calculated based on the quantiles in the first percentile and the 99th percentile. This method was used to filter the samples to ensure the nFeature\_RNA data was between the lower and upper bounds. Although the upper and lower bounds calculated provided a range for the data, it may not have accurately filtered all of the unwanted cells.

## SingleR Cluster Analysis

The SingleR analysis of the gene clusters did not provide significant information about the cellular differences between the AD and control samples. As shown in Figure 5A, the AD samples expressed astrocytes, B cells, chondrocytes, dendritic cells, embryonic stem cells, endothelial cells, fibroblasts, gametocytes, hepatocytes, HSC\_G-CSF, HSC\_CD34+, macrophages, monocytes, neuroepithelial cells, neurons, NK cells, osteoblasts, platelets, smooth muscle cells, T cells and tissue stem cells. Figure 5B of the control samples highlighted the same cells except for chondrocytes. Overall, both samples highly expressed astrocytes, monocytes and neuroepithelial cells. Since both samples were taken from the brain, it is assumed that they will express neuronal cells regardless of the disease state. Further, the only difference in cells found between the AD samples and the control samples were chondrocytes, which are cells which produce cartilage and maintain homeostasis within joints [17]. This does provide some insight into the disease state as certain AD mechanisms of chronic immune hyperactivity can lead to osteoporosis and cause cell death in chondrocytes [6]. As a result, the AD samples expressing chondrocytes may be due to the post-mortem patients experiencing osteoporosis at the same time of the disease.

Further, gametocytes and hepatocytes that were highlighted by the SingleR analyses are in-

consistent with cells that are found within the brain. Specifically, gametocytes such as oocytes are found in reproductive organs of females and are not found in the brain region [12]. Hepatocytes are found in the liver and functions to complete oxidative metabolism through processes such as gluconeogenesis, amino acid catabolism and bile formation [10]. It is not possible for these cells to be found within the brain regions and as a result, SingleR cannot be the only analysis method used to make conclusions about the cell types in the AD and control samples. A key issue may have been the Human Primary Cell Atlas Data reference dataset the clusters were compared to, which may not have had a large subset of brain sample data. Thus, in the future, SingleR analysis can be completed with other reference datasets to determine the most accurate cellular compositions of the samples.

## Conclusion

Overall, the purpose of this study was to determine the major marker genes from 2 human samples of AD patients in Braak stages three and four and 2 human samples of control brains. Through Seurat cluster analysis, it was determined that the AD samples had major marker genes of ADAM28, ZNF148, FLT1, CSF3R, LPAR5, CTNNA3, and SLC1A2 among others. While the control samples had major marker genes of CUX2, NORAD, RFN219-AS1, GRB14, SEMA3E and VCAN. Both samples exhibited ENPP2 and DOCK8 genes as well. In addition, SingleR analysis methods were not ideal for this investigation as it did not provide accurate representations of the data. The analysis method also highlighted gametocytes and hepatocytes in the samples however this was inaccurate as generally these cells cannot be found within these regions. As such, in future studies SingleR can be further investigated to determine accurate means to cluster data. Thus, through single-cell RNA sequencing, the major marker genes in AD patients and their respective roles in the disease were determined. This provides a broader understanding of gene functionalities associated with AD.

# Appendix

## Pre - Processing Data Analysis

The following figures outline the pre-processing plots that were created throughout the analysis process prior to creating UMAPS and completing cluster analyses.

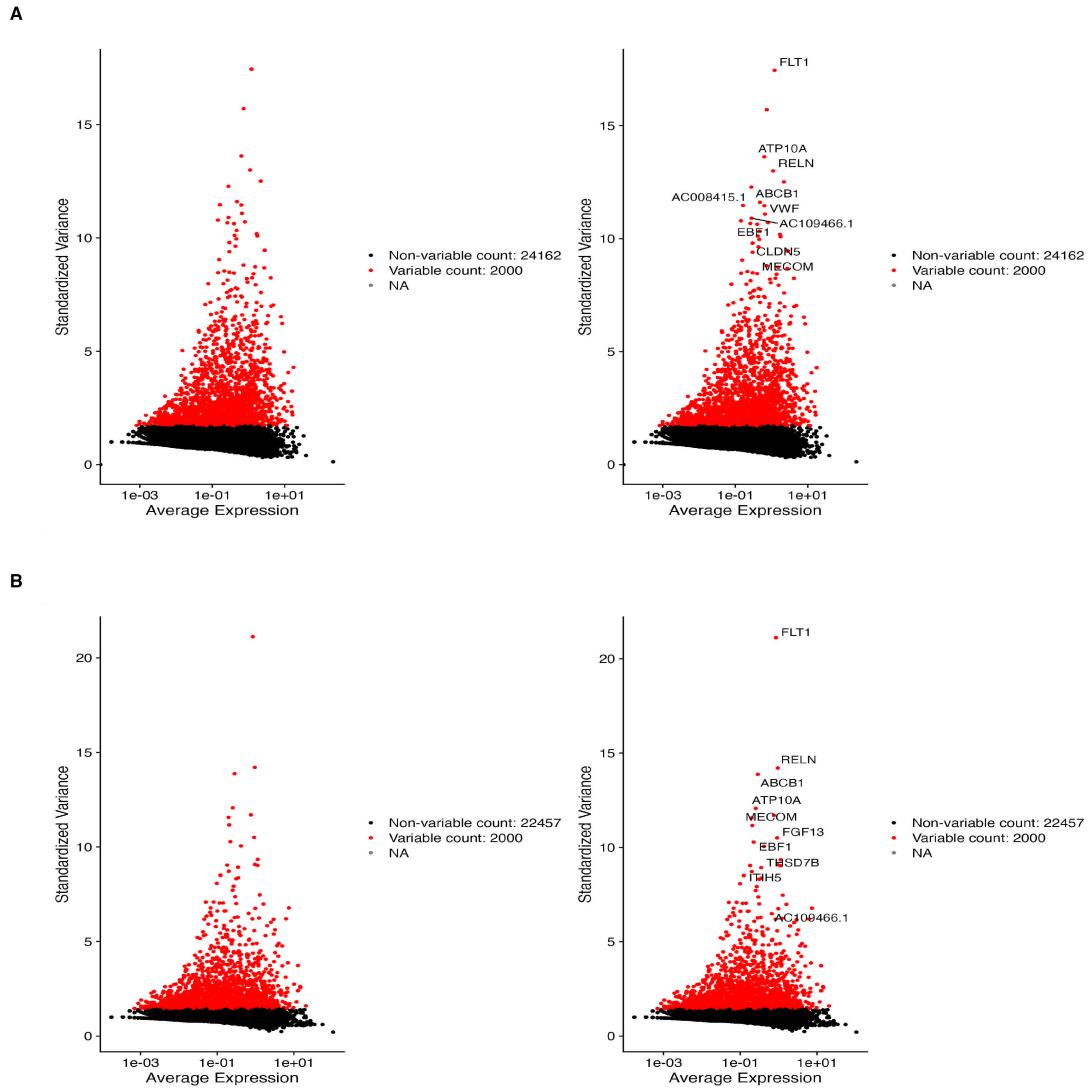


Figure 6: Highly Variable Features – Feature Selection

The top 10 most highly variable genes were determined, and the variable feature plot function was utilized in order to determine the top features for the AD data (A) and control data (B).

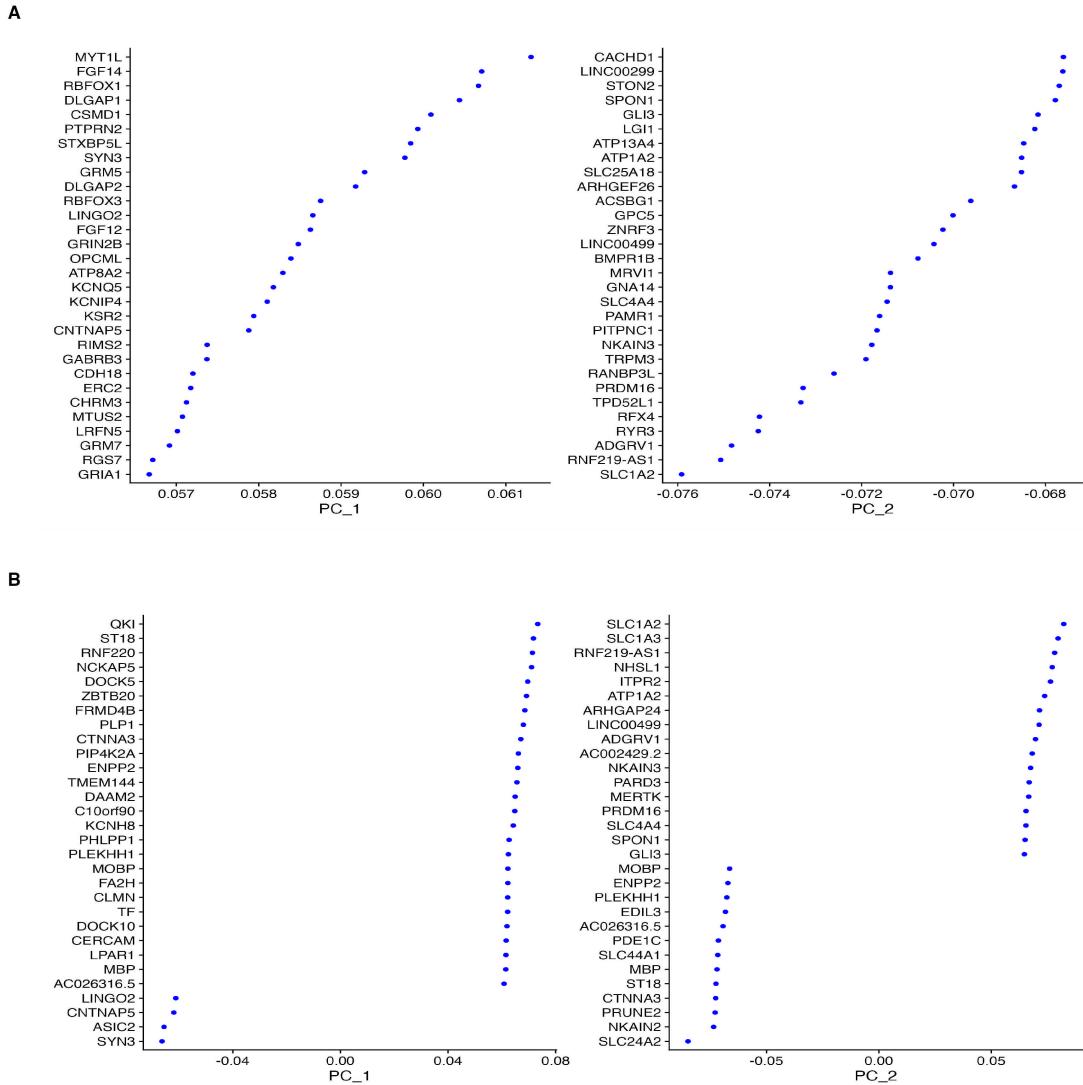


Figure 7: Linear Dimensional Reduction Plots

After the datasets were scaled, linear dimensional reduction was completed and PCA was conducted. The above VizDimLoadings plot described the top genes which are associated with reduction components for the AD data (A) and control data (B).

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