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Computational Analysis of Cell Type-Specific Transcriptional Dynamics in Major Depressive Disorder

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Abstract. Depression, also known as major depressive disorder (MDD), is a complex mental health condition that affects millions of people worldwide. Since disease treatments often depend on levels of understanding, this project strives to learn more about the transcriptional components of MDD to delineate the transcriptomic dynamics in MDD. The sample includes the single-cell transcriptomic profiles in the dorsolateral prefrontal cortex (dlPFC) of 34 postmortem patients. This data was acquired from publicly available datasets and run through R-Studio code. Cell clusters were classified into cell types using cell-type-specific signature markers in the original resource datasets. After analyzing immediate early genes (IEGs), MDD-associated genes, and differentially expressed genes, the trend showed broadly lowered expression levels and frequency in MDD patients than in healthy control with some exceptions. After studying 10 different IEGs, the trend showed a lowered gene expression frequency in MDD patients than in control. For MDD-associated genes, a similar trend showed lower frequency and expression levels in the MDD sample, apart from GAD1 and RELN, which had a higher expression level in the MDD sample. Then, by analyzing the top differentially expressed genes in each cell type, most cells showed a lowered frequency in MDD patients besides macrophage/microglial, which had higher expression frequency in IGHG1 and RP11-315A16.1 for the control sample. With each analytical finding, the underlying elements of MDD may be better understood, leading to more precise wet lab experiments and the eventual treatment of MDD.

Keywords: Major depressive disorder (MDD); Dorsolateral prefrontal cortex (dlPFC); Transcriptomic dynamics.

1. Introduction

Depression, also known as major depressive disorder (MDD), is a complex mental health condition that is prevalent in nearly a fifth of adults in the U.S. [1]. According to DSM-5, a notable book used globally for mental health, symptoms may include a consistently depressed mood, diminished pleasure in many activities, weight loss/gain, loss of energy, feelings of worthlessness and guilt, and thoughts/plans of suicide [2]. Though the mental and physical effects of MDD are broadly known, there is yet to be a foolproof way to treat MDD. Many existing medications have adverse side effects. For example, the two most commonly used antidepressants, SSRIs and SNRIs, may cause agitation, insomnia, and sexual dysfunction [3]. The brain region most associated with MDD is the prefrontal cortex (PFC), which is generally linked with cognitive function, regulating thoughts, actions, and emotions [4]. Additional evidence has shown that the alterations in structure, markers of neurotransmitters, and connectivity within the PFC are associated with MDD [5]. To find better treatments for MDD, understanding the underlying molecular components would be necessary. Changes in transcriptomics may provide insights into the underlying mechanisms of MDD [6, 7]. Transcriptomics studies existing mRNA in cells, gauging the gene expression in cells. Transcriptomics is useful for detecting how certain diseases affect gene expression. In the case of depression, little research has been done on individual cells. As the brain is highly varied in cell types, bulk sequencing is less effective in determining gene expression profiles at cell-specific levels.

Immediate early genes (IEGs) are genes that are expressed readily in response to numerous signals. They play a big role in brain development, learning, and responses and are essential in understanding the effects of external conditions on the transcription in a body [8]. Research has shown that the

prefrontal cortex was noticeably lacking in activity by examining IEGs, including ZIF268, FOS, and ARC, within a postmortem human brain [9]. Similarly, when conducting the experiment on mice, the same reductions in IEG expression were found in the PFC [10].

A recent study uses single-cell RNA sequencing (scRNA-seq) to document cell type-specific transcriptional adaptations across the dlPFC in 34 post-mortem human samples [11]. The sample comprises 17 MDD patients and 17 control samples. This study laid the foundation for the transcriptional analysis of IEGs and MDD-associated genes in each cell type. Numerous studies have been conducted to correlate specific gene expression levels with MDD. To better understand the cellular mechanisms involved, IEGs and MDD-associated genes are analyzed to find a cell type-specific expression in dlPFC. Moreover, the goal aimed to identify the top differentially expressed genes in each characterized cell type in the dlPFC of MDD and control groups using differential expression analysis methods. The newly identified differentially expressed genes in each cell type will provide potential new insights into the molecular basis of MDD.

2. Materials and Methods:

2.1. List of materials

(1) Raw single-cell RNA-sequencing data generated by the 10X Genomics platform was downloaded from the Gene Expression Omnibus under dataset GSE144136, and a copy of the dataset was stored, containing "GSE144136_GeneBarcodeMatrix_Annotated.mtx.gz", "GSE144136_GeneNames.csv.gz", and "GSE144136_CellNames.csv.gz."

(2) Personal computer, RStudio, R Packages (Seurat, Tidyverse, readr, ggplot2, patchwork)

(3) Internet access for downloading datasets and code.

2.2. Experimental Procedures

(1) The sample consists of the 78,886 nuclei extracted from postmortem human brain tissue within the BA9 region of the prefrontal cortex from 17 control subjects and 17 MDD subjects who died by suicide. All subjects were male with an average age of 38-41, but further information was kept hidden for confidentiality. The scRNA dataset was imported and loaded into Seurat, and "Seurat" was used in all analyses thereafter. The cells were determined to be pre-filtered after running a test to check for mitochondrial DNA.

(2) The preprocessing was performed to normalize cell data. The Seurat command, "SCTransform", normalized the data and detected high-variable genes. The top 3,000 (default number) genes will be used in Principle Component Analysis, tSNE, and UMAP dimensionality reductions. To begin clustering, anchors were set up for data integration and the elimination of batch effect. Then, the most similar cells were clustered using "FindClusters" with a resolution of 1.5, resulting in 42 initial clusters named 0-41 using the function.

(3) Classifying each cluster to various cell types by cell markers. Cell marker information was obtained from the original source material. 42 initial clusters were categorized into 6 different cell types: excitatory neurons (Excitatory), inhibitory neurons (Inhibitory), Astrocytes (Astro), macrophages and microglia (Macrophage/Microglial), oligodendrocytes (Oligo), oligodendrocyte progenitor cells (OPC). Unmatched clusters exhibiting various genes were recognized to be double droplets and were removed from the dataset.

(4) IEG and MDD-associated gene expression pattern analysis. IEG gene names and MDD-associated gene names were retrieved from PsyGeNET and DisGeNET. Expression levels and frequencies were measured in 6 identified cell clusters. Dot plot and Violin Plot were created to compare the differences between MDD and control patients.

(5) Differentially expressed genes analysis. To search for highly differentiated genes in each of the 6 cell types, the function "FindAllMarkers" was used to identify marker genes between MDD and control samples. Dot plots were created to show the top differentially expressed genes.

3. Results

3.1. Cell type-specific expression of IEGs in MDD

The cell type selective expression of IEGs *ARC, FOS, FOSB, JUN, JUNB, JUND, BDNF, EGR1, EGR3,* and *EGR4* was investigated using present single-cell RNA-seq datasets of the dlPFC. These gene expressions on the dataset between MDD patients and healthy control samples were then examined. Based on the dot plot of the IEG expression pattern (Figure 1), Numeric data is available upon request), most IEGs show a lower expression frequency and higher average expression. Average expression is the percent of all cells that express a particular gene, while percent expressed is the expression frequency within a cell.

All IEGs showed a lower expression frequency in MDD than the control sample, while most of the IEGs had higher gene expression levels (indicated with red dots) in the control group than in the controls. The results indicate that IEGs are expressed in fewer cells but may be enriched in these cells in MDD cells. Since IEGs are signatures of cell activity, it can be inferred that cells broadly were deactivated in MDD, but enrichment of IEG expression in IEG-expressing cells may be related to the pathology of MDD.

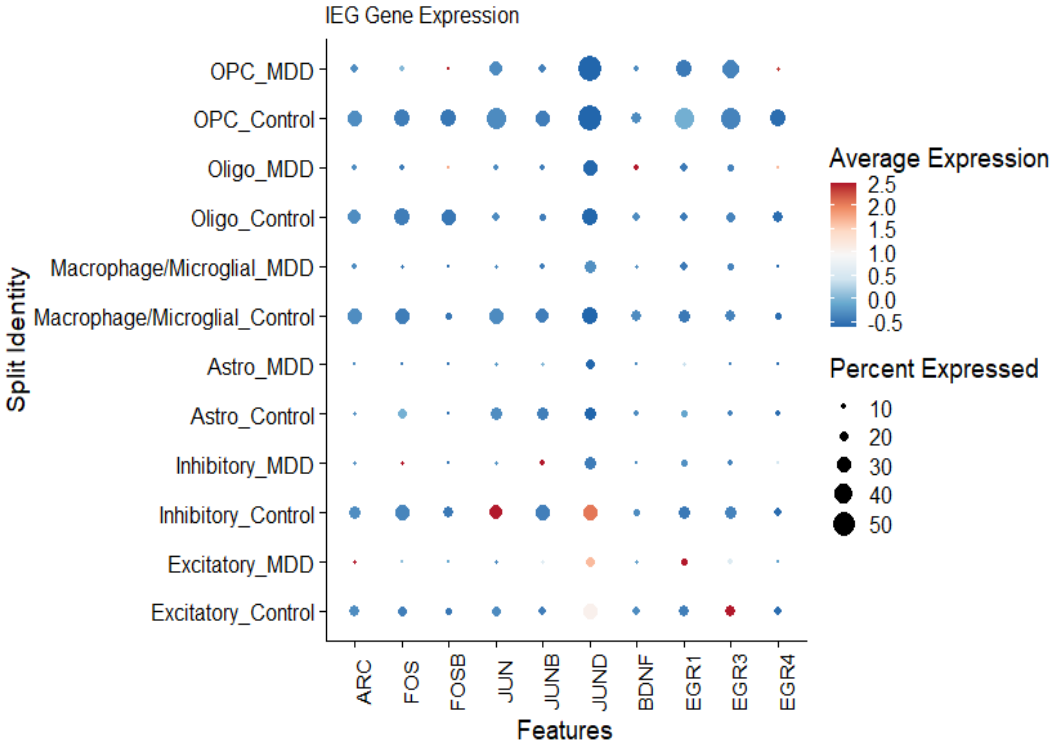


Figure 1. Dot plot showing the cell-type specific expression of IEGs differentiated between MDD patients and control samples

3.2. MDD-associated gene expression

To examine MDD-associated gene expression, top MDD-associated genes were taken from the public gene databases PsyGeNET and DisGeNET. Each database has its own type of scoring system determined by different algorithms (more information in DisGeNET and PsyGeNET). With the MDD-associated genes identified, the goal was to examine cell-type-specific gene expression frequency and level (Figure 2). The data concludes that *HSPA1B, HSPA1A, S100A10,* and *EDN1* have consistently lower gene expression levels and frequency in MDD patients than in the control

group. Comparatively, *GAD1* has a similar expression frequency in all cell types except the astrocytes, which have a lower expression frequency in MDD patients. In oligodendrocyte progenitor cells, the expression level of *GAD1* increased, as indicated by the red coloring. Similar to *GAD1*, data from *RELN* shows similar expression levels and frequency in nearly all cells aside from astrocytes, which have higher expression frequency. The astrocytes in MDD patients have high *RELN* expression levels, but similar expression frequency as control samples.

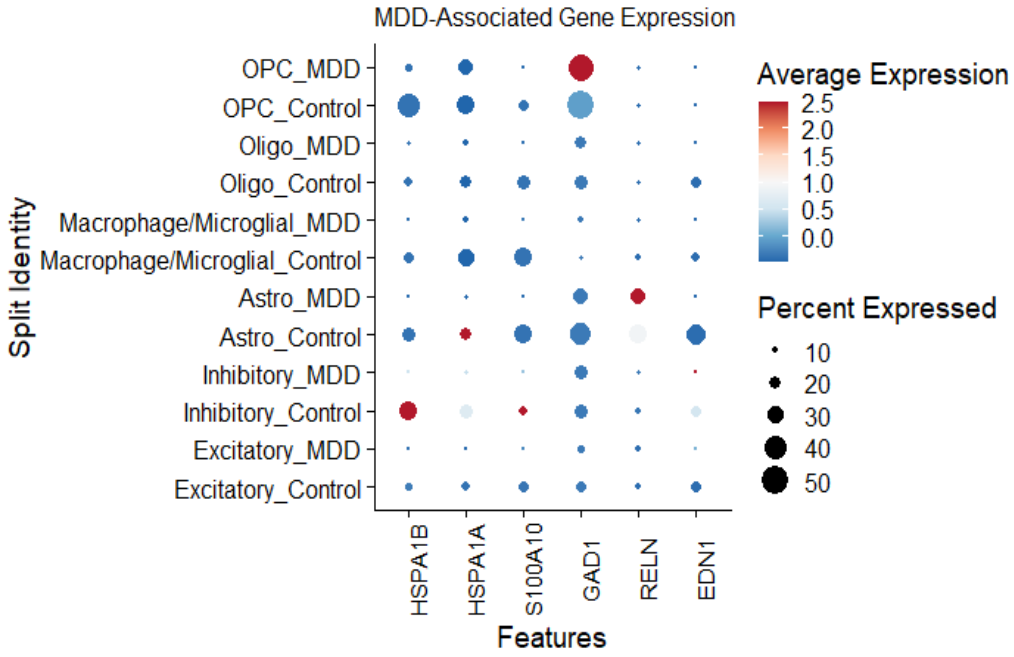
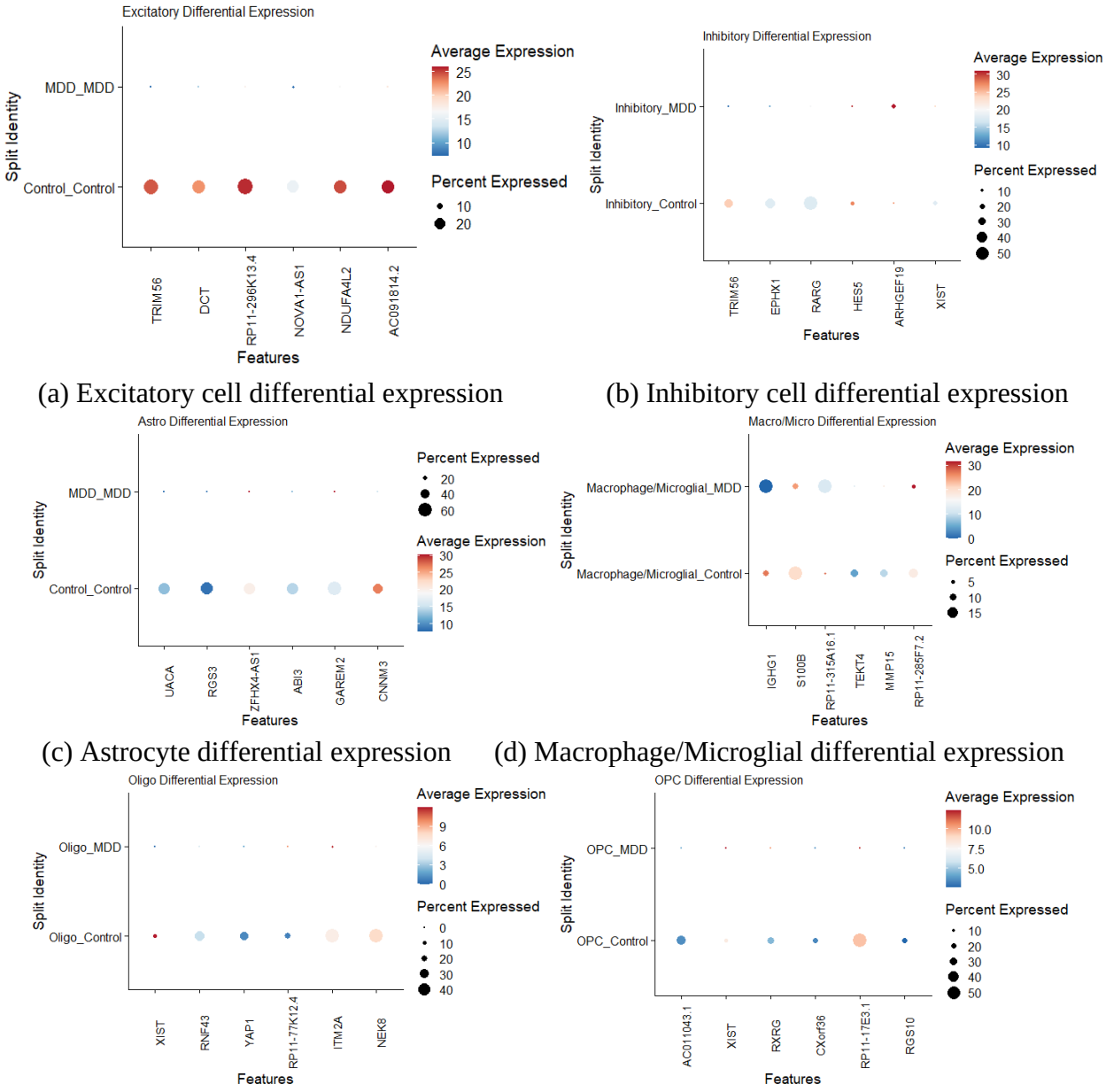


Figure 2. MDD-associated gene expression in cell-type-specific genes

3.3. Differentially Expressed Genes in Each Cell Type

The top differentially expressed genes were studied in each characterized cell type in dlPFC in 34 samples using differential expression analysis methods. The top 6 most varied genes across two groups for each cell type were identified (Figure 4).

By analyzing the dot plots, the trend revealed that the control group generally had a higher expression frequency than the MDD sample, particularly in astrocytes. Of the 1576 control astrocytes, around 51% expressed differentially expressed genes, while in 2496 MDD astrocytes, around 2% expressed these genes (Numeric data is available upon request). Inhibitory cells, oligodendrocytes, and oligodendrocyte progenitor cells expressed mixed results, with some genes having higher expression frequency in the control than the MDD sample, while others had similar expression frequency in both samples. In macrophage/microglial cells, the genes *S100B*, *TEKT4*, *MMP15*, and *RP11-285F7.2* have higher expression frequencies in the control group than in the MDD patients. On the contrary, *IGHG1* and *RP11-315A16.1* have higher expression frequencies in MDD samples than in control.



(a) Excitatory cell differential expression (b) Inhibitory cell differential expression

(c) Astrocyte differential expression (d) Macrophage/Microglial differential expression

(e) Oligodendrocyte differential expression (f) Oligodendrocyte progenitor cells differential expression

Figure 3. The top differentially expressed genes in dlPFC cell types across 34 samples

4. Discussion

By conducting computational analysis of publicly accessible single-cell RNA sequencing datasets [11], the broad clusters of cells were determined and evaluated to check for IEG expression, MDD-associated gene expression, and differential expression. Furthermore, the genes that exhibited the most significant differential expression in each cluster were found, providing insight into the genes that are most affected by MDD. It is necessary to study the genes with the largest difference between MDD and control since the difference implies an alteration due to MDD. In contrast, similar expression frequencies and levels signify maintained homeostasis despite the effects of MDD. By analyzing the cell-type-specific differences in gene expression levels and frequency, it not only finds the genes that are differentially expressed in control and MDD patients but also locates the types of cells most vulnerable to MDD. Because this project can locate both the types of cells and genes MDD effects, it paves a more efficient pathway for wet labs to proceed while verifying results from dry labs. For instance, optogenetics, which studies the individual cells' function in a system using light stimulation, would benefit greatly from studies that locate cell-specific changes due to diseases.

(1) After running the experiment, the data showed that IEG expression is lower in MDD patients than in control. Similar to the results of previous studies in humans and mice, the depressive PFC exhibits lower levels of IEGs like *ARC and FOS* [9]. Because of the expression difference in the two samples, it is especially necessary to study these genes. On the other hand, the exceptions listed should also be further studied to understand the underlying mechanisms that would cause those genes to be overexpressed in MDD patients.

(2) The results of analyzing MDD-associated genes show a lowered expression level and frequency in all genes except *GAD1* and *RELN*. The genes *HSPA1B, HSPA1A, S100A10,* and *EDN1* show a lower expression frequency in MDD patients than in control, denoting MDD's effect on specific genes. These results remain consistent with previous findings for the genes *GAD1* and *RELN*.

GAD1 encodes one of the glutamic acid decarboxylase enzymes, which plays a role in gamma-aminobutyric acid (GABA) formation from glutamate. According to the GABAergic Deficit Hypothesis of MDD, the neurotransmitter, GABA, plays an essential role in the etiology of MDD [12]. Another study considered *GAD1*'s relation to MDD and other psychiatric disorders and found that variations in *GAD1* may affect a person's susceptibility to MDD [13].

RELN, the gene that codes for the protein reelin, has been previously studied to find its potential association with suicide and depression. In the end, the single nucleotide polymorphism between the rs2965087 was found to be associated with suicide threats [14].

(3) Differentially expressed genes showed a lower gene expression frequency in over half of the selected genes. Most other genes remained constant, while only *IGHG1* and *RP11-315A16.1* had higher expression frequency in MDD patients than in control. Since the abnormal expression in these genes occurred in macrophage/microglial cells, the trend implies a connection to MDD. Regarding macrophages, deep research has been conducted connecting macrophages with neuroinflammation, which potentially causes MDD [15]. Furthermore, microglia regulate inflammation, neural connections, and synaptic plasticity, which all relate to MDD [16].

Additionally, astrocytes in MDD cells were observed to have a lower gene expression frequency compared to the control group. The lowered astrocyte expression indicates a degeneration in astrocyte functions. Astrocytes are associated with neurotransmitter take-up, axonal growth, and the regulation of the blood-brain barrier [17]. Other research has been done regarding astrocytes and the blood-brain barrier, though it is unclear whether dysregulation is a factor that causes MDD or a consequence of MDD [18].

With 36 new biomarkers, scientists have the potential to detect MDD, potentially saving millions of dollars in MDD medication.

5. Conclusion

MDD leads to many epigenetic and transcriptional alterations in the dlPFC, suppressing or increasing the expression levels of certain genes. To better understand the cellular mechanisms involved in MDD pathology, it is necessary to question whether IEGs, such as *FOS, JUN, and ARC,* exhibit a cell type-specific expression in the dlPFC. As found in the analysis, patients with MDD expressed a lowered expression frequency for the 10 IEGs studied in this project, showing lower cell activity. In the MDD-associated genes, the overall trend showed lowered expression levels and frequency aside from *GAD1* and *RELN*, which showed similar expression frequency and a higher expression rate in particular cells. After doing research on these two genes, it was determined that the variance may be due to *GAD1* and *RELN*'s role in the nervous system. Finally, this experiment studied differentially expressed genes, which found lower expression frequencies in nearly all cell types, especially astrocytes. The only exceptions to this trend were from the *IGHG1* and *RP11-315A16.1* genes in macrophage/microglial, which may indicate an association between immunological health and MDD. The specific cell-type expression of the dlPFC based on the computational analysis might be relevant to designing potential new pharmaceutical and therapeutic approaches to tackle MDD.

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