Differential Gene Expression Analysis of Alzheimer's Disease in the Entorhinal Cortex Using GEO2R

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by memory decline, synaptic dysfunction, and neuronal loss. The Entorhinal Cortex (EC) is one of the earliest brain regions affected during AD progression and plays a crucial role in memory processing and neural connectivity.

Transcriptomic analysis enables the identification of Differentially Expressed Genes (DEGs) associated with disease mechanisms. By comparing gene expression profiles between Alzheimer's patients and healthy controls, molecular pathways involved in neurodegeneration can be identified.

The objective of this study was to identify differentially expressed genes between Alzheimer's disease and control samples in the Entorhinal Cortex using publicly available transcriptomic data from the Gene Expression Omnibus (GEO) and analyzed with the web-based tool GEO2R.

2. Methods

2.1. Dataset Selection

The dataset used in this study was **GSE5281**, obtained from the NCBI Gene Expression Omnibus (GEO). This microarray dataset contains gene expression profiles from multiple brain regions of Alzheimer's patients and healthy controls.

To avoid confounding effects from different brain regions, only samples from the **Entorhinal Cortex (EC)** were selected.

Sample distribution:

- Alzheimer's disease (EC_Affected): 10 samples
- Healthy controls (EC_Control): 13 samples

Total samples analyzed: 23

2.2. Group Definition

Samples were grouped as follows:

- Group 1 (Alzheimer): EC_affected samples
- Group 2 (Control): EC_control samples

2.3. Differential Expression Analysis

Differential expression analysis was performed using **GEO2R**, which applies the **limma (Linear Models for Microarray Data)** statistical framework.

Parameters used:

- Multiple testing correction: **Benjamini–Hochberg (False Discovery Rate)**
- Significance threshold:
  - Adjusted p-value < 0.05
  - |log2 Fold Change| ≥ 1

2.4. Reproducibility

The analysis was performed three times:

1. Replication 1 – Standard parameters (adj.p < 0.05, |log2FC| ≥ 1)
2. Replication 2 – Repeated with identical parameters
3. Replication 3 – More stringent threshold (adj.p < 0.01)

Across all replications, gene expression trends remained consistent, demonstrating robustness and reproducibility of the analysis workflow.

3. Results

3.1. Identification of Differentially Expressed Genes

Out of **54.675 analyzed genes**, a total of:

- **5.665 significant DEGs** were identified
  - **2.567 upregulated genes**
  - **3.089 downregulated genes**

The higher number of downregulated genes suggests widespread transcriptional suppression in the Entorhinal Cortex during Alzheimer's disease.

3.2. Examples of Significant Genes

Representative upregulated genes include:

- **GFAP** – astrocyte activation marker
- **C1QA** – complement pathway component
- **CD68** – microglial activation marker
- **HLA-DRA** – immune response gene
- **TYROBP** – regulator of microglial signaling

These genes are strongly associated with neuroinflammation.

Representative downregulated genes include:

- **SYN1** – synaptic vesicle regulation
- **CAMK2A** – synaptic plasticity
- **GRIN1** – NMDA receptor subunit
- **ATP5F1A** – mitochondrial ATP production
- **SNAP25** – neurotransmitter release

These genes are essential for neuronal signaling and synaptic function.

3.3. Volcano Plot Visualization

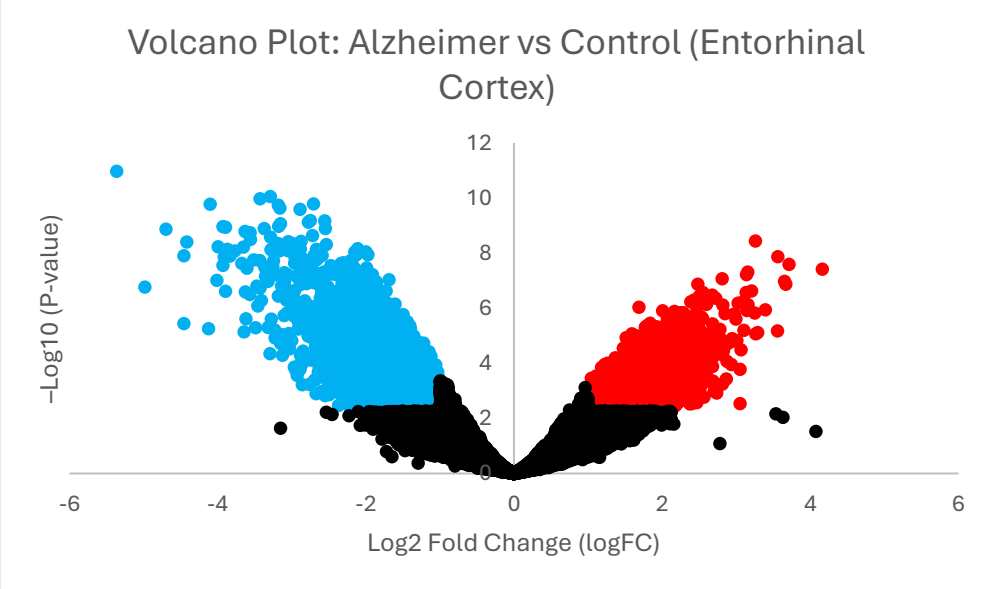

Figure 1. Volcano plot of differential gene expression in the Entorhinal Cortex comparing Alzheimer's disease and control samples.

The X-axis represents log2 Fold Change (log2FC), and the Y-axis represents –log10 P-value. Significantly upregulated genes are shown in red, significantly downregulated genes in blue, and non-significant genes in black.

The volcano plot demonstrates:

- A substantial number of significantly downregulated genes (blue) on the left side
- A large number of significantly upregulated genes (red) on the right side
- Non-significant genes (black) clustered near the center

The broader distribution of downregulated genes is consistent with the higher count of suppressed genes (3.089) compared to upregulated genes (2.576), indicating prominent neuronal transcriptional repression in Alzheimer's disease.

4. Biological Interpretation

The upregulation of immune-related genes supports the well-established role of neuroinflammation and microglial activation in Alzheimer's disease pathogenesis.

Meanwhile, the downregulation of synaptic and neuronal genes reflects:

- Synaptic dysfunction
- Impaired neurotransmission
- Reduced mitochondrial activity
- Progressive neuronal degeneration

These findings align with known pathological mechanisms of Alzheimer's disease, particularly in the Entorhinal Cortex, one of the earliest affected regions.

5. Conclusion

This study successfully identified 5,665 differentially expressed genes in the Entorhinal Cortex of Alzheimer's patients compared to healthy controls using GEO2R.

Key findings include:

- Extensive transcriptional dysregulation
- Predominance of downregulated neuronal genes
- Upregulation of immune and inflammatory pathways
- Reproducible results across three analytical replications

The analysis confirms that Alzheimer's disease is associated with widespread molecular alterations involving neuroinflammation and synaptic impairment.

GEO2R proved to be a reliable and accessible web-based tool for preliminary transcriptomic differential expression analysis.