Evaluating the Role of ALS Subtypes in the Choroid Plexus and their Influence on Clinical Outcomes

Jordan Garcia[1], Jarrett Eshima[2], Barbara Smith[1]

[1]School of Biological and Health Systems Engineering, Tempe, AZ

[2]Airobes LLC, Phoenix, AZ

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurological disorder characterized by the progressive degeneration of motor neurons, leading to muscle weakness, paralysis, and eventually death within 2-5 years of symptom onset[1]. Despite significant research efforts, predicting disease progression remains a major challenge due to the complex and poorly understood clinical heterogeneity. Recent advancements in omic technologies and bioinformatic approaches have provided new tools for improving ALS diagnosis and predicting disease progression[2]. By analyzing transcriptomic data from large patient cohorts, researchers have begun to identify different disease patterns that help classify ALS into distinct molecular subtypes. These include glial activation (ALS-Glia), oxidative stress (ALS-Ox), and transcriptional dysregulation (ALS-TD), which may each play a role in ALS progression. Building on previous studies in the motor cortex and spinal cord, we investigated the presence of these molecular subtypes within the choroid plexus, a critical yet understudied brain structure involved in cerebrospinal fluid production and CNS homeostasis[3,4]. Enrichment analyses further reveal subtype-specific biological pathways involved in neurodegeneration and allow for correlation with clinical outcomes, offering new insights into ALS pathogenesis and progression.

Aim

This study aims to investigate the molecular subtypes of ALS by analyzing transcriptomic data from the choroid plexus of 64 individuals. Through unsupervised clustering and enrichment analysis, we seek to uncover distinct gene expression patterns associated with clinical outcomes, offering new insights into ALS progression.

Choroid Plexus

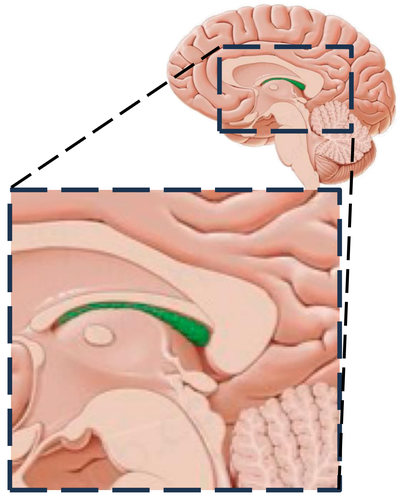


Figure 1: Choroid Plexus Location. The choroid plexus is a specialized structure in the brain responsible for producing cerebrospinal fluid (CSF) and maintaining the blood-CSF barrier[4].

Methods

Human Subjects - This study included RNA-seq data from 56 ALS patients and 8 healthy patients. Patient data included information on sex, age at symptom onset, age at death, ethnic background, and collection site.

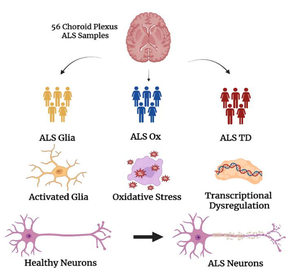


Figure 2: 56 total ALS subjects

TE Profiling:
- 18,678 transposable elements identified
- Differential expression analysis performed

Clustering:
- NMF via SAKE to define TE-based subtypes

Pathway Enrichment:
- GSEA & Enrichr used to find subtype-specific pathways

Clinical Correlates:
- Survival: Kaplan-Meier + log-rank test
- Age analysis: ANOVA with post-hoc & FDR correction
- Comorbidities: Chi-squared test

Results

Unsupervised Clustering

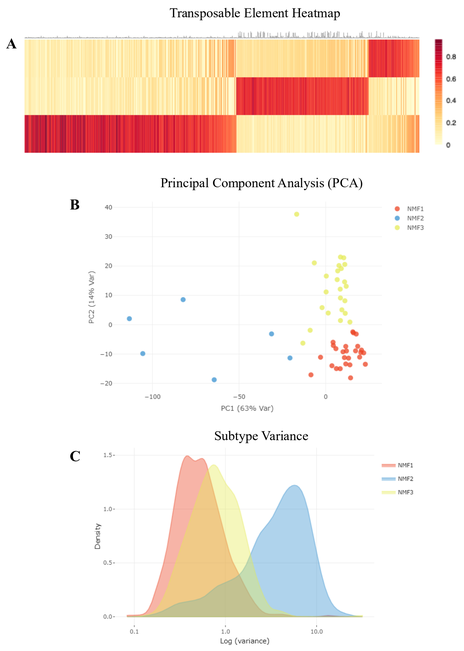


Figure 3: Unsupervised clustering analysis of ALS transcripts. **A** Transposable Elements heatmap. **B** Principal component analysis (PCA) shows three distinct clusters of ALS. **C** The three distinct clusters of ALS separated based on levels of variance

Enrichment Analysis

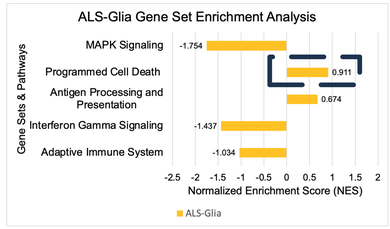


Figure 4: ALS-Glia Gene Set Enrichment Analysis. Pathway analysis revealed upregulation of immune activation and neural cell death signaling, implicating glial-driven neuroinflammation and motor neuron damage in ALS-Glia patients.

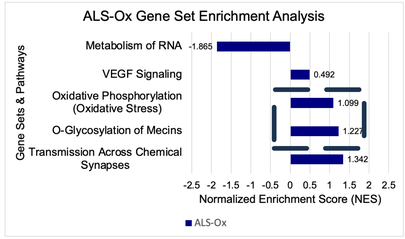


Figure 5: ALS-Ox Gene Set Enrichment Analysis. ALS-Ox patients exhibited enrichment in oxidative phosphorylation and synaptic signaling pathways, alongside upregulated O-glycosylation, suggesting metabolic adaptation and potential links to extended survival.

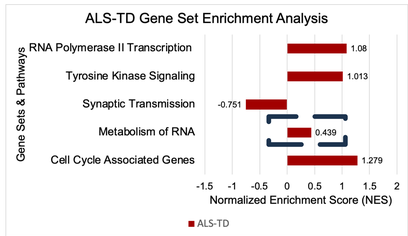


Figure 6: ALS-TD Gene Set Enrichment Analysis. ALS-TD subtype showed enrichment in RNA metabolism and retrotransposon-related pathways, highlighting transcriptional dysregulation as a key molecular feature.

Survival & Clinical Outcomes

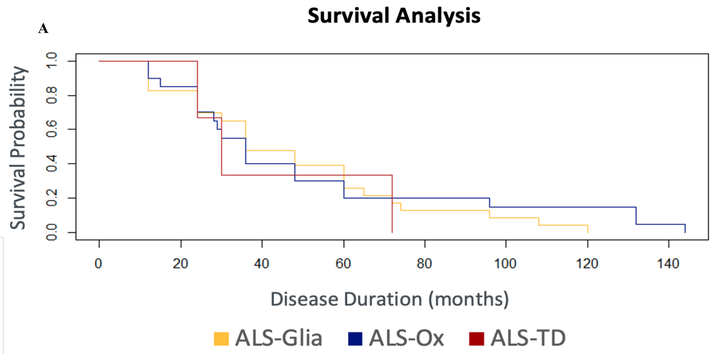
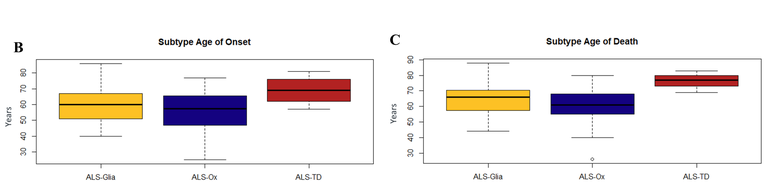


Figure 7: Assessment of ALS patient clinical parameters. A Kaplan-Meier survival analysis reveals significant differences in disease duration across ALS subtypes, with ALS-Ox exhibiting the longest survival and ALS-TD the shortest. **B** Age of disease onset plotted as boxplots for three ALS subtypes. A box-and-whisker plot is used to show the highest median age of onset occurs within the ALD-TD subtype while the shortest median age of onset occurs within the ALS-Ox subtype. **C** Age of disease death plotted as boxplots for three ALS subtypes. A box-and-whisker plot shows the highest median age of death occurs within the ALD-TD subtype while the shortest median age of onset occurs within the ALS-Ox subtype.



Summary, Conclusions and Future Directions

This study identified three distinct molecular subtypes of ALS, including ALS-Glia, ALS-Ox, and ALS-TD, within the choroid plexus using transcriptomic profiling and patient stratification. Each subtype displayed unique gene expression signatures and was associated with different clinical outcomes, particularly survival duration and age of disease onset. These findings highlight the role of immune signaling, oxidative stress, and transcriptional regulation in ALS progression and underscore the potential of molecular subtyping in improving prognosis.

Future Work:
Future work will focus on validating these subtypes in larger, independent cohorts and exploring their relevance in other brain regions. Continued investigation may support the development of personalized treatment strategies tailored to specific molecular profiles.

References

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Acknowledgements

I am sincerely thankful to Jarrett Eshima for his generous and invaluable guidance during the analysis process, as well as for granting me the opportunity to build upon his foundational research, which served as the cornerstone of this project. I would also like to express my deepest gratitude to my parents for their constant support and encouragement. Most of all, I dedicate this project to my Tata, Manuel Garcia. Your strength and pride have always inspired me. I hope I've made you proud.