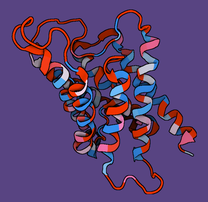
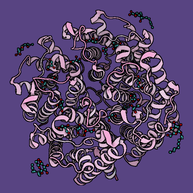


snRNA Transcriptomic Analysis to Assess the Role of AQP1 and AQP4 as Biomarkers for Duchenne Muscular Dystrophy



Hugo Jal Hernández

INTRODUCTION

While genetic mutations in **dystrophin** are well-documented, a gap remains in understanding the involvement of aquaporins (AQPs) in DMD pathology. Specifically, **AQP4**, which regulates water transport in **skeletal muscle**, has been implicated due to its reduction in DMD-affected muscle and its association with **dystrophin-associated protein complex** (DAPC) instability.

Previous research has lead to hypothesis regarding **AQP1's upregulation** as a potential **compensatory** mechanism to **AQP4's downregulation** in Duchenne muscular dystrophy.

However, the role of AQP4 and AQP1 in DMD **remains ambiguous**, with inconsistent findings across studies.

METHODS

In this project, **single-nucleus RNA sequencing** (snRNA-seq) data from **VL muscle samples** from both healthy and DMD patients was analyzed. Data was provided as a preprocessed Seurat object, which included prior steps like quality control, normalization, and scaling.

The analysis focused on identifying **changes in cell type proportions**, with a reduction in myofibers and an increase in adipocytes observed in DMD samples. **Differential gene expression analysis**, particularly of aquaporins (AQP1 and AQP4), was conducted using the **Wilcoxon Rank Sum test**. Then, **enrichment analysis** of differentially expressed genes identified relevant **pathways**.

Correlation matrices for AQP1 and AQP4 were generated to explore **gene associations**, further supported by pathway enrichment to relate aquaporin function to **clinical features** of DMD.

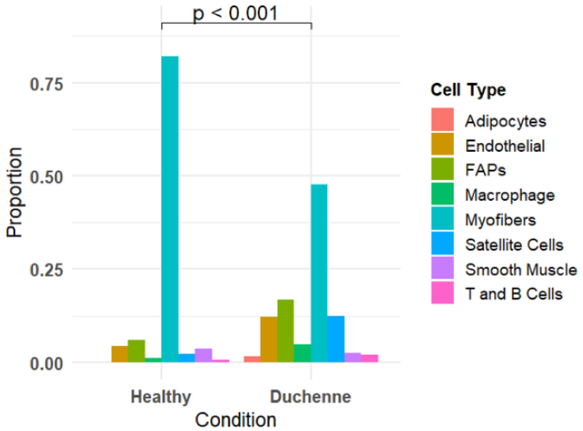
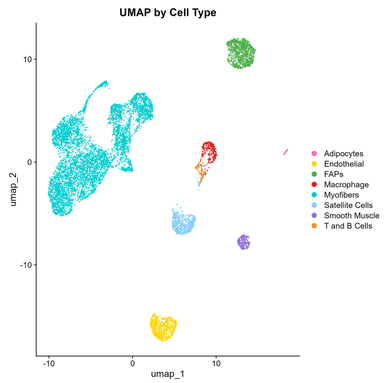


Figure 1: Barplot showing the differing cell type proportion by condition status.

RESULTS



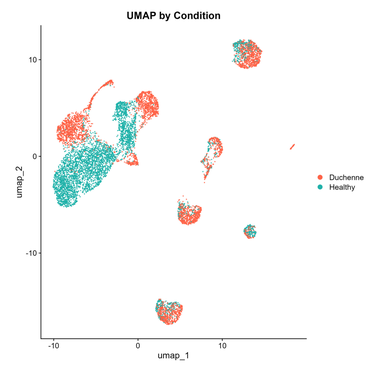
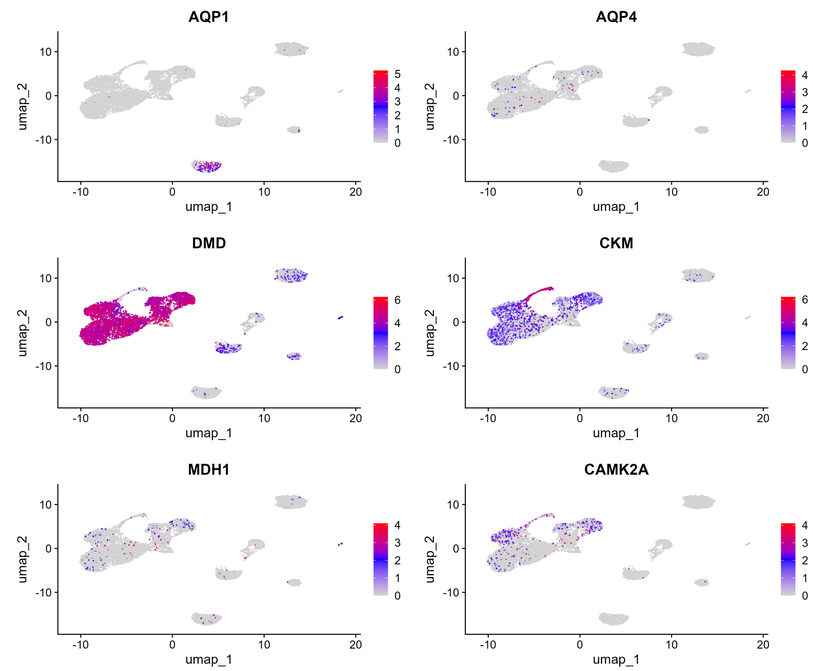


Figure 2: UMAP visualization illustrating population idetification and healthy vs. diseased cells.



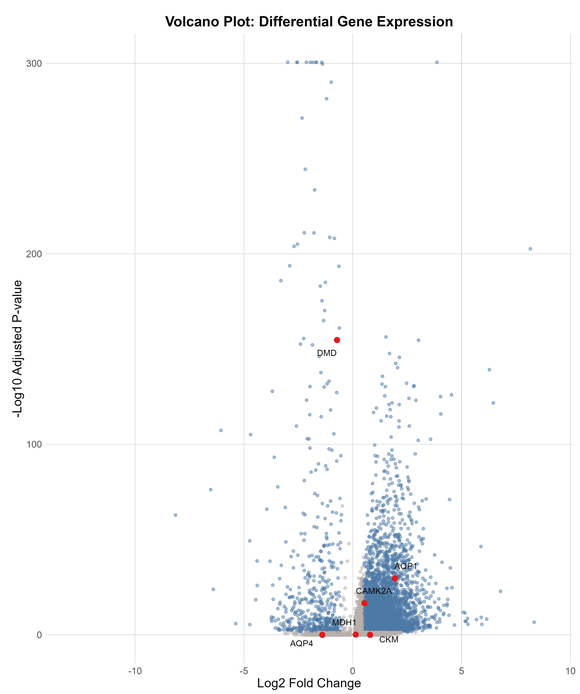
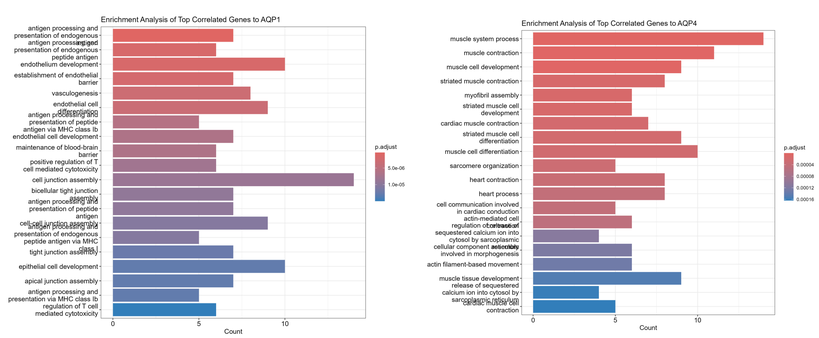


Figure 3: Volcano plot showing the results of differential expression analysis. Genes of interest in red.

Figure 4: UMAP visualizations illustrating genes of interest and where they are expressed.
Figure 5: Barplots showing which pathways were enriched in the subsets of highly correlated genes to AQP1 (left) and AQP4 (right) respectively.



CONCLUSIONS

Summary

- Differential expression analysis showed **significant upregulation of AQP1**.
- While **AQP4** exhibited a **trend towards downregulation**, though not statistically significant.
- Pathway enrichment analysis highlighted significant **alterations** in **extracellular matrix organization**, **cytoskeleton dynamics**, and **inflammation-related pathways**, supporting known DMD pathology.

The contrasting expression patterns of AQP1 and AQP4 **suggest their relevance in DMD pathophysiology**, particularly in muscle repair and water homeostasis, warranting **further multiomics studies** for deeper exploration .

Figure 6: Heatmap of top 50 differentially expressed genes.

Gene	p_value	avg_log2FC	pct.1	pct.2	adjusted_p_value	FDR_BH
DMD	< 0.001	−0.7223411	0.553	0.824	< 0.001	< 0.001
CKM	0.1148	0.7954821	0.318	0.291	1	0.1292
AQP1	< 0.001	1.942773	0.066	0.015	< 0.001	< 0.001
AQP4	0.0647	−1.401034	0.018	0.024	1	0.0745
MDH1	< 0.001	0.1364925	0.061	0.041	0.7070	< 0.001
CAMK2A	< 0.001	0.5384431	0.118	0.059	< 0.001	< 0.001

Table 1: Results for differential expression analysis of genes of interest.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the members of the Nelson Lab at UCLA for generously sharing their dataset with me. Their collaboration and support have been invaluable to my research, enabling me to explore new insights into Duchenne muscular dystrophy. I appreciate their commitment to advancing scientific knowledge and their willingness to contribute to my work. Thank you for your assistance and encouragement throughout this process.

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