ANALYSIS OF

DRD3 GENE EXPRESSION

In Essential Tremor Patients with Relation to Gender & Age

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INTRODUCTION

Essential Tremor (ET) is disorder of the nervous system primarily characterized by rhythmic shaking. To begin our exploration of ET, we reviewed a study conducted by researchers at Columbia University Medical Center. Their findings concluded ET is a family of related disorders brought about by various types of biological dysfunction[1]. The scope of our research evolved and we pivoted to explore a single gene of interest, Dopamine D3 receptor (DRD3), found to be linked to ET patients.

UNSUPERVISED TECHNIQUES REVEAL AGE GROUP AS VARIABILITY SOURCE IN DATA

These techniques were completed to reduce the dimensionality of the dataset. Began with unsupervised hierarchical clustering (Figure 1) to create categories, mapped with principal component analysis (Figure 2) to visualize the leads with some form of variability explained by clusters, and completed a box plot (Figure 3) to verify these age group categories exist.

Cluster Dendrogram

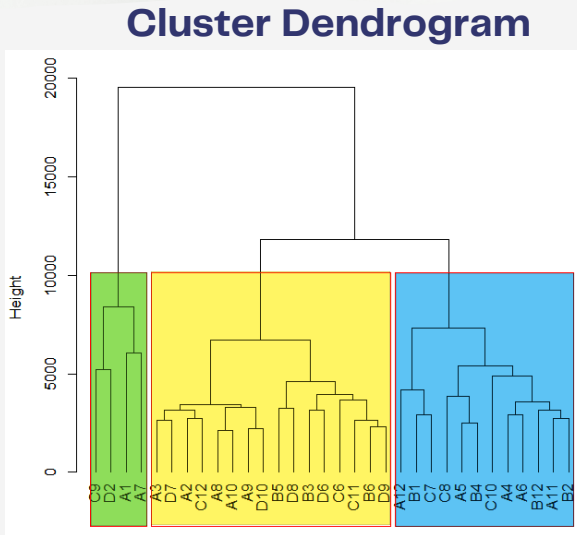


Figure 1: Cluster Dendrogram created with unsupervised clustering reveals three groups

PCA Plot with Respect to Age

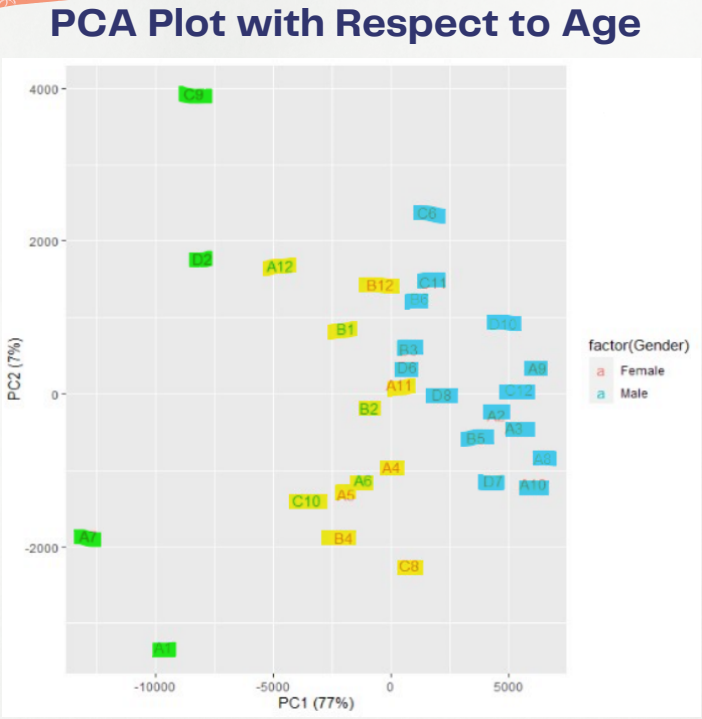


Figure 2: Groups defined from unsupervised clustering algorithm color-coded to the dendrogram. Some contribution in variability to clustering visible.

Box Plot of Age Groups

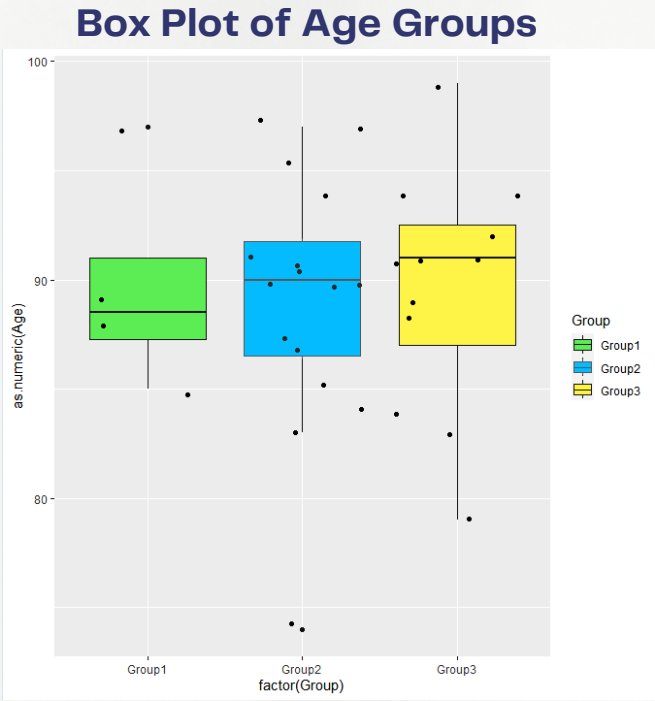


Figure 3: Slight increase in age in groups from left to right. This suggests that age is a weak contributor to variability, but there may be other factors at play.

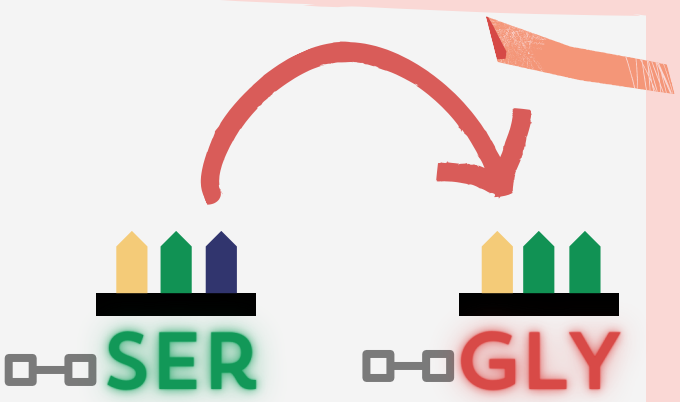
No Differentially Expressed Genes with Respect to Gender

Following differential analysis, we concluded that there are no differentially expressed genes with respect to gender. The closer the logFC is to zero, the less of a difference between gene expression of control vs. ET. Since all genes show logFC close to 0, there are no differentially expressed genes with respect to gender. All Wald tests show $p > 0.05$ so we cannot reject the null hypothesis.

CHOOSING DRD3 AS OUR GENE OF INTEREST

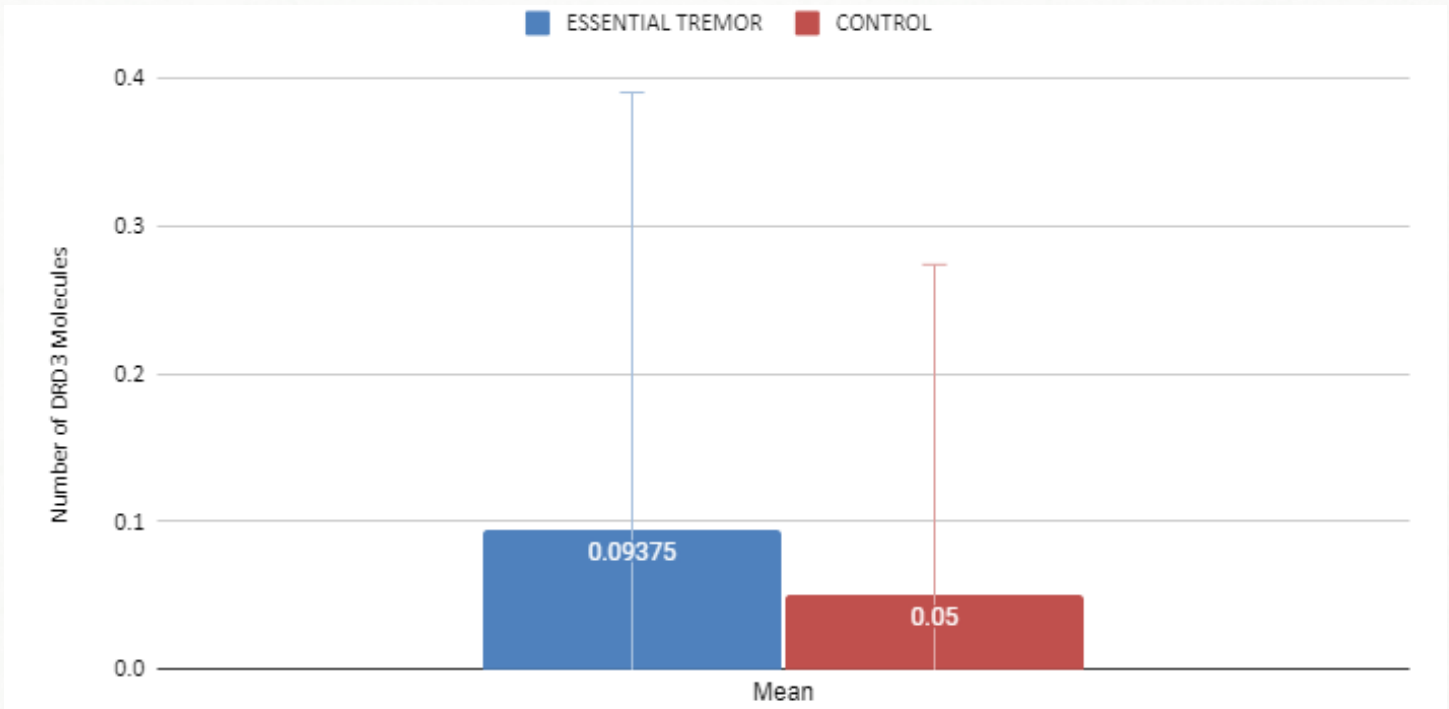
We identified our gene of interest based on a genome wide linkage scan of Icelandic families, where a linkage peak marker of the ETM1 locus emerged and was located from 1 to 10 mega-bases away from a DRD3 mutant.

This mutant variant of DRD3 (rs6280) is believed to be positively associated with Essential Tremor development[2]



Point mutation on serine residue on position 9 of the N terminus in DRD3 causes glycine replacement.

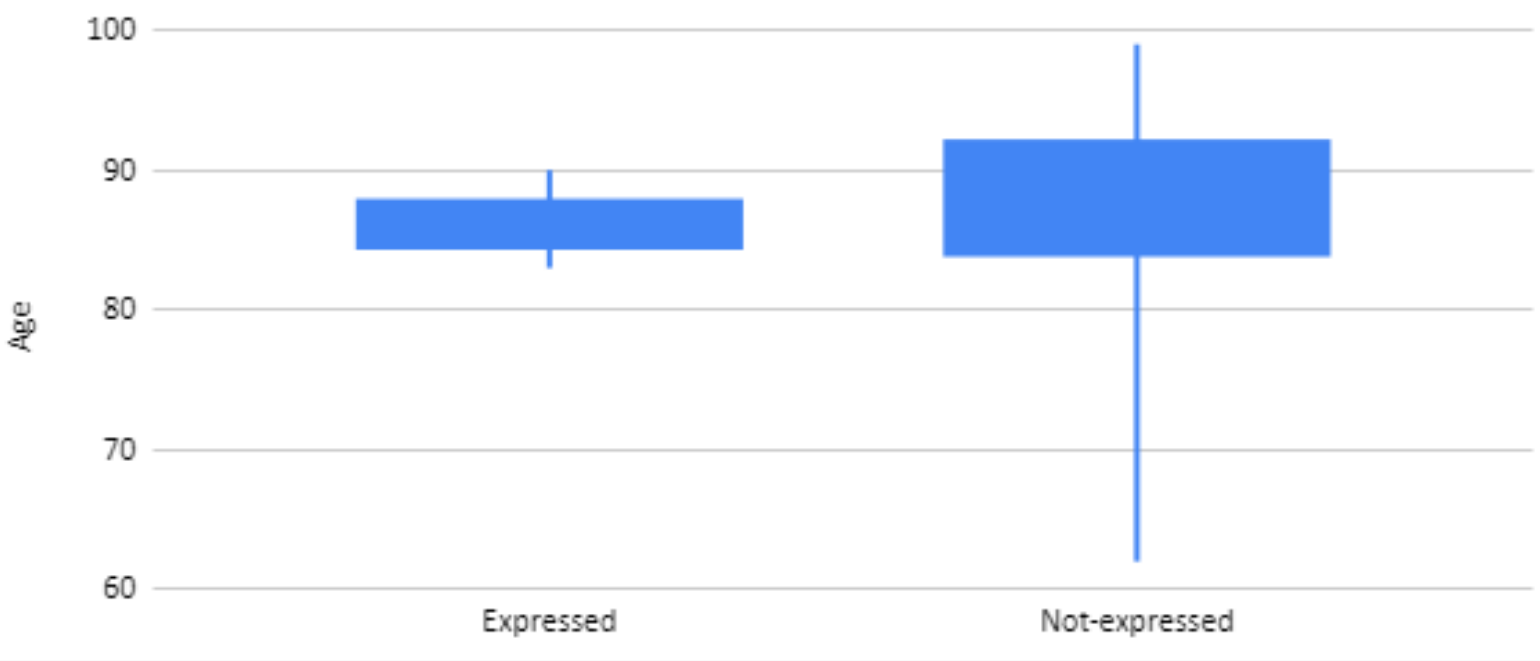
ANALYSIS IN ET VS. CONTROL



DRD3 Expression in ET vs Control

Comparing the mean gene expression level (number of DRD3 molecules expressed) between ET samples (n = 32) and control samples (n = 20), a student t-test yields 0.57 (> 0.05). Therefore we cannot reject the null hypothesis that there is a statistically significant difference between the gene expression level of DRD3 between ET and control groups.

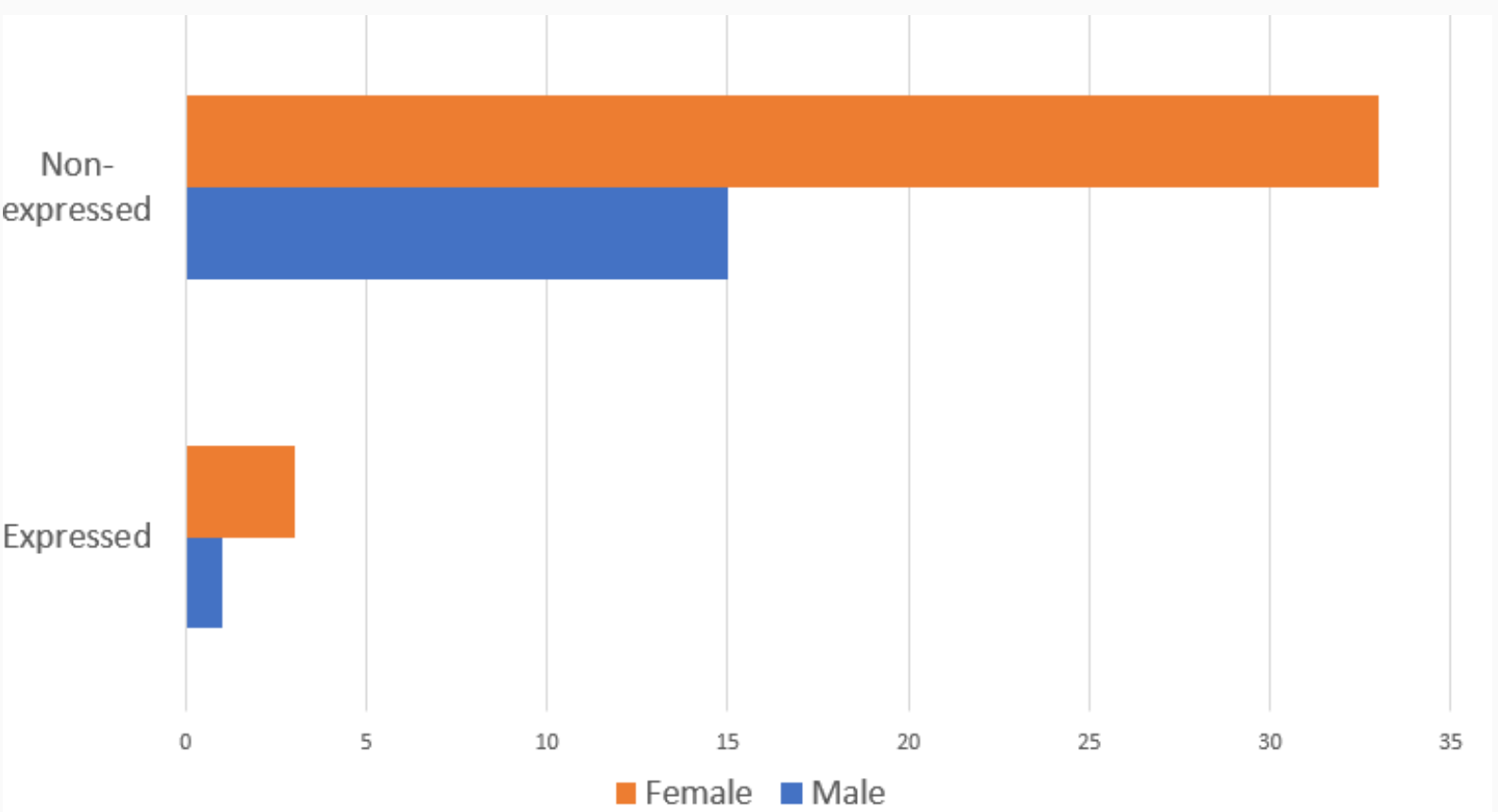
ANALYSIS IN RELATION TO AGE



Box Plots of Age Distribution in DRD3-expressed vs Non-expressed

DRD3-expressing samples have age distribution on the lower end compared to DRD3-non-expressing samples, though still fitting within the Quartile 1- Quartile 3 range of the DRD3-non-expressing samples

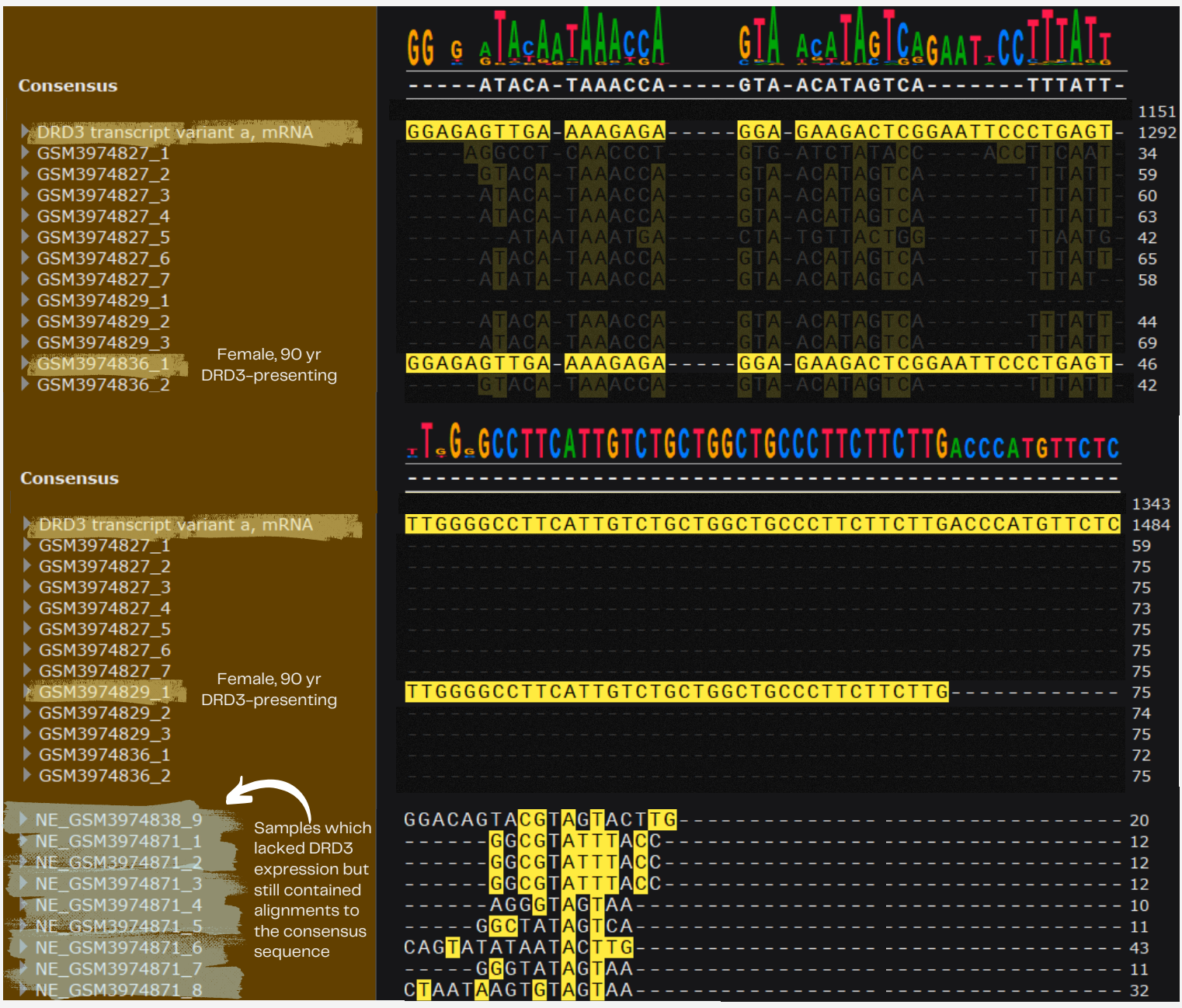
ANALYSIS IN RELATION TO GENDER



Expression and Lack of Expression of DRD3 Relative to All Patients

A bar graph showcasing DRD3 expressing and non-expressing samples in relation to patient gender. The ratio of DRD3-presenting females to males matches the general ratio more overall female patients in the sample set. A Chi-Squared test showed these results to be not statistically significant: (X2 = 0.0625, **p-value = 0.80 > 0.05**).

GENE ALIGNMENTS & ANALYSIS



Alignments were completed in Snapgene utilizing the MUSCLE algorithm, aligning mRNA fragments to DRD3 isoform a (NM_000796.6). It is possible to see only one fragment of one sample (DRD3-presenting, Female, 90; GSM3974829) aligns best to the consensus sequence compared to the remaining samples. Even the control samples without identified DRD3 expression show expression of mRNA, suggesting that these samples require further purification.

CONCLUSIONS & FUTURE DIRECTIONS

We did not identify any significant clustering pattern in the PCA plot, dendrogram, and box plot with respect to age/age groups, but there does appear to be association to age groups. We also did not identify any differential expressed genes with respect to gender. Gene alignment was inconclusive as well due to the small size of the mRNA fragments of DRD3. Additional clean-up of the RNA-Seq data used would be required to form one true mRNA sequence to compare to the consensus. Our findings did overall corroborate that of the original study, where 'gene hits' of interest were found, but did not exhibit increased expression given any age group or gender.

ACKNOWLEDGEMENTS

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WORKS CITED

1. Martuscello, R. T., Kerridge, C. A., Chatterjee, D., Hartstone, W. G., Kuo, S. H., Sims, P. A., Louis, E. D., & Faust, P. L. (2020). Gene expression analysis of the cerebellar cortex in essential tremor. Neuroscience letters, 721, 134540. https://doi.org/10.1016/j.neulet.2019.134540

2. Siokas, V., Aloizou, A. M., Tsouris, Z., Liampas, I., Aslanidou, P., Dastamani, M., Brotis, A. G., Bogdanos, D. P., Hadjigeorgiou, G. M., & Dardiotis, E. (2020). Genetic Risk Factors for Essential Tremor: A Review. Tremor and other hyperkinetic movements (New York, N.Y.), 10, 4. https://doi.org/10.5334/tohm.67