Computational Biology Approach to Identifying the Genetic Pathways Affecting Neurodegenerative Diseases

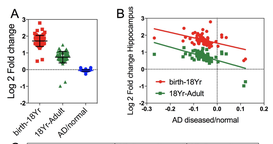
Project SBI118

Introduction

- Neurodegenerative diseases, such as **Alzheimer's, Frontotemporal Dementia, and Huntington's Disease**, kill neurons over time, resulting in the loss of a specific function
- They are **irreversible and can be devastating**. They are expensive to treat and often, the treatment is ineffective. Many of the root causes underlying neurodegenerative disease are unknown, and currently there is no cure for the vast majority. These diseases overwhelmingly effect the aging population; due to increased life expectancy, millions of people are affected and there is an **urgent need to discover the root of these diseases and find potential cures**
- Over 5.5 million Americans of all ages had Alzheimer's Disease, as of 2016; estimated 5.3 million people aged 65+
- If we could find genes that affect the development of the function that is lost and **also** play a role in the disease, **we could find the causes and thus the cures of these diseases**

Previous (2017) Work

- Goal - Find if there are **genes that affect both the function of memory as the hippocampus develops, and Alzheimer's**; if they exist, find what patterns those genes exhibit in healthy and diseased brains.
- Procedure - I used the Hippocampus as the experimental data and the Cerebellum as the control data
- Results - **I found 74 significant genes. Genes whose expressions changed in the hippocampus during development are deregulated in the Alzheimer's affected hippocampus.**



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	AD diseased/normal vs. birth-18Yr	AD diseased/normal vs. 18Yr-Adult
r	-0.4785	-0.5715
95% confidence interval	-0.6373 to -0.2807	-0.7076 to -0.3945
R squared	0.229	0.3266
P value		
P (two-tailed)	<0.0001	<0.0001

A: The x-axis of this graph shows the hippocampus in various stages: before birth/18yrs (red), 18yrs/adult (green), and between Alzheimer's-affected and normal hippocampi (blue).

B: A graph showing correlation of the genes that change in development (before birth/18yrs, red) and in adulthood (18 yrs/adult, green) with the x-axis showing the genes that changed in an Alzheimer's affected hippocampus. This graph reveals an inverse relationship.

C: Tabular results of correlation analysis.

Research Question & Hypothesis

- Inspired by last year's findings, the purpose of this project was to continue my research into neurodegenerative diseases from last year, to expand its scope.
- **My expanded research question was:** Will all neurodegenerative diseases have developmental genes that are deregulated?
- **My global hypothesis,** by extension, was that genes that change in a specific region of the brain but not in the cerebellum, during development will be deregulated in that same region when it is affected by a certain neurodegenerative disease.

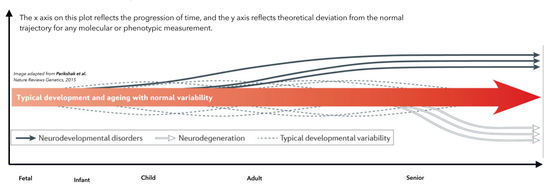
Disease	Experimental	Control
Alzheimer's	Hippocampus	Cerebellum
Frontotemporal Dementia	Frontal Cortex	Cerebellum
Huntington's	Striatum	Cerebellum

Research & Related Work

A genetic correlation between neurodegenerative disease and brain development is a novel idea, and is the basis of my work.

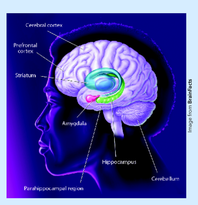
Neurodevelopmental vs. Neurodegenerative Diseases. In general, neurodevelopmental and neurodegenerative diseases have been seen as two distinct categories of brain disorders. There has been no correlation between the genes that change during brain development and during disease.

- **Neurodevelopmental diseases** are caused by issues in the regulation of fundamental processes in the development of the brain, such as ADHD and autism.
- **Neurodegenerative diseases** result in neuron death.



Analysis by Brain Regions. Neurodegenerative diseases affect distinct regions of the brain. However, the underlying reasons for this specificity in neurotoxicity are unknown. I found significant genes for each disease by comparing affected region to the cerebellum, because the cerebellum remains unaffected in most neurodegenerative diseases.

- **Alzheimer's Disease.** Mainly leads to neuronal death in the **hippocampus**, which results in **memory loss**.
- **Frontotemporal Dementia.** Affects the **frontal cortex** of the brain. The main function affected by FTD is higher **cognitive abilities**.
- **Huntington's Disease.** Mainly affects the **striatum**, whose function is **mobility**. It can have symptoms such as **involuntary movements**.



Procedure & Statistical Analysis

Step 1: Prepare Allen Atlas Human Brain data for analysis
Download gene expression data and group data into expressions from each region.
Divide data into age groups.

Step 2: Statistical Analysis on AA Data
Run statistical analyses for all genes in each region, then select significant genes (all p-values < .05 AND only significant in experimental region)

Step 3: Prepare Gene Expression Omnibus data for analysis
Download gene expression data and group data into Diseased and Healthy

Step 4: t-test on Gene Expression Omnibus data
Run t-tests for all genes using Diseased vs. Healthy groups, then select significant genes (p-value < .05)

Step 5: Compare Allen Atlas and Gene Expression Omnibus data
Compare significant genes found in both major groups to complete project goal

Step 6: GSEA (Gene Set Enrichment Analysis)
See what pathways/ functions significant genes perform for verification

Repeat for each disease

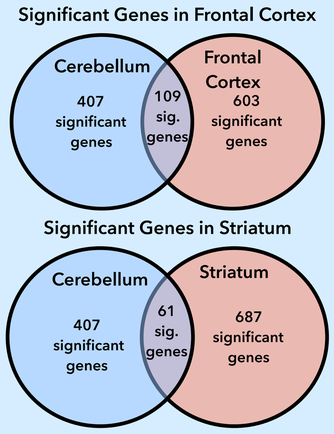
Step 2: Statistical Analysis of Allen Atlas Data

- I used **MATLAB** to run a one way **An**alysis **o**f **Va**riance (ANOVA) test on each gene. An ANOVA test compares the means of each sample to generate a p-value.
- **Three age groups** to ensure even distribution of existing samples in dataset
  - Fetal: Before birth
  - Infant & Child: Birth-18 years
  - Adult: 19+ years old
- I used **multcompare with Bonferroni correction** to find p-values when comparing the age groups pairwise:
  - A multcompare in MATLAB compares two datasets, generating a p-value.
  - A Bonferroni correction is a statistical correction done to avoid a Type 1 error, or a "false positive", by dividing a p-value by the number of tests done.
- **Gene significant if:**
  - p-value from ANOVA < 0.05
  - pairwise p-values after Bonferroni correction are < 0.05.

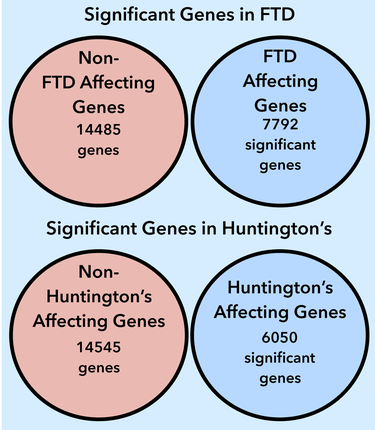
Step 4: Statistical Analysis of Gene Expression Omnibus Data

- I used a **t-test** to analyze the Gene Expression Omnibus data. A two-sample t-test compares two datasets—here, the datasets are the data from the diseased brains, and the data from the healthy brains. I did not divide the data into age groups because I only wanted to see how genes were changed through disease. Thus, a t-test was sufficient to analyze this data, unlike the statistical analysis for the Allen Atlas data, where I used an ANOVA test to compare three age groups.

Step 2: Statistical Analysis on AA Data

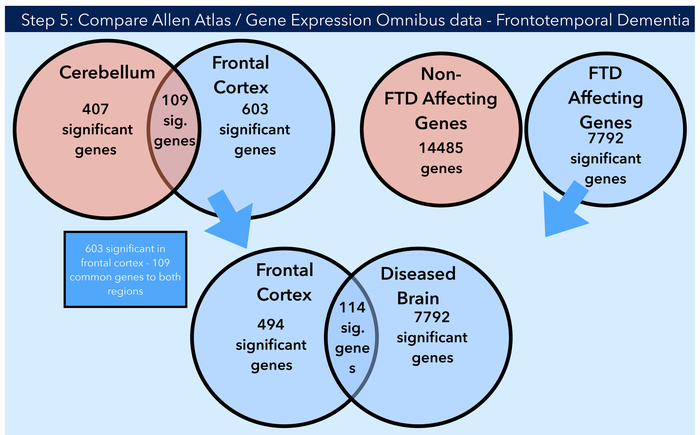


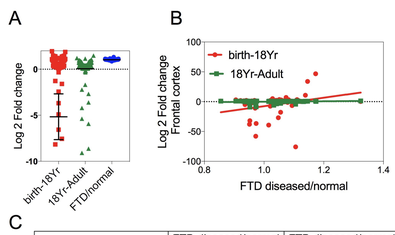
Step 4: T-Test on Gene Expression Omnibus data



Results

Step 5: Compare Allen Atlas / Gene Expression Omnibus data - Frontotemporal Dementia





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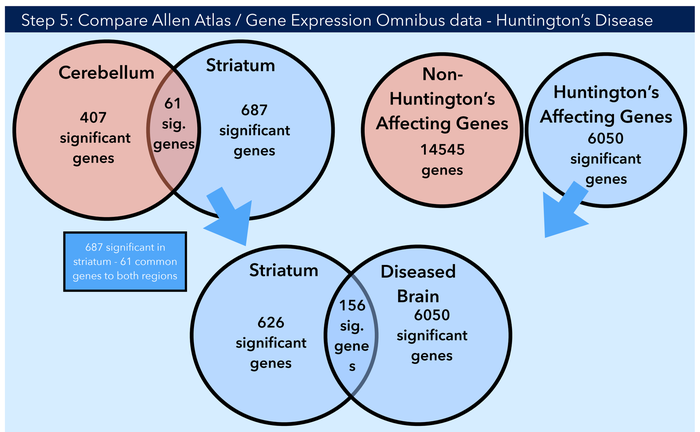
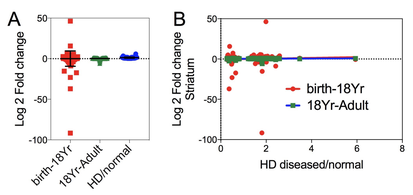
	FTD diseased/normal vs. birth-18Yr	FTD diseased/normal vs. 18Yr-Adult
Pearson r		
r	0.1751	0.1535
95% confidence interval	-0.009061 to 0.3479	-0.03135 to 0.3281
R squared	0.03068	0.02355
P value		
P (two-tailed)	0.0623	0.1031

- **Genes whose expressions changed in the frontal cortex during development show no correlation to those in the FTD affected frontal cortex.**
  - **A:** The x-axis of this graph shows three groups of Log2 fold changes: the frontal cortex before birth/18yrs (red), 18yrs/adult (green), and between FTD-affected and normal frontal cortices (blue).
  - **B:** A graph showing correlation of the genes that change in development (before birth/18yrs, red) and in adulthood (18 yrs/adult, green) with the x-axis showing the genes that changed in an FTD affected frontal cortex. Again, the Log2 fold ratios are shown here.
  - **C:** Tabular results of correlation analysis.
- Graph B plots the fold ratios of each significant gene in this disease. The green points represent the fold ratio for the expressions in the adult brain, and the red for the childhood and fetal brains. They are plotted against the fold ratios in the diseased brains. Here, **no strong correlation is observed**. Unlike in the Alzheimer's graph, there is no strong upward or downward trend, showing statistical insignificance.

Step 5: Compare Allen Atlas / Gene Expression Omnibus data - Huntington's Disease





C

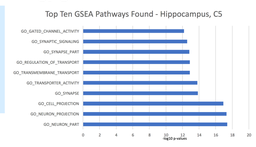
	HD diseased/normal vs. birth-18Yr	HD diseased/normal vs. 18Yr-Adult
Pearson r		
r	0.0377	0.1002
95% confidence interval	-0.1202 to 0.1937	-0.05784 to 0.2534
R squared	0.001421	0.01004
P value		
P (two-tailed)	0.6403	0.2133

- **Genes whose expressions changed in the striatum during development show no correlation to those in the Huntington's affected striatum.**
  - **A:** The x-axis of this graph shows three groups of x Log2 fold changes: the striatum before birth/18yrs (red), 18yrs/adult (green), and between Huntington's-affected and normal striatum (blue).
  - **B:** A graph showing correlation of the genes that change in development (before birth/18yrs, red) and in adulthood (18 yrs/adult, green) with the x-axis showing the genes that changed in an Huntington's-affected striatum. Again, the Log2 fold ratios are shown here.
  - **C:** Tabular results of correlation analysis.
- Similar to Frontotemporal Dementia, there is no major trend observed in Graph B, which plots the fold ratios of each significant gene in this disease. Again, the green points represent the fold ratio for the expressions in the adult brain, and the red for the childhood and fetal brains. They are plotted against the fold ratios in the diseased brains.

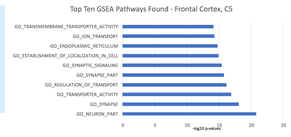
GSEA Results

Gene Set Enrichment Analysis (GSEA) is a way to **retrieve a functional profile of a given gene set** in order to better understand the **underlying biological processes.** For each structure, I used two gene sets to categorize the pathways of the significant genes identified in Step 5: **C2 Curated gene sets** and **C5 Gene Ontology gene sets.** Shown below are some of the top pathways found in the **C5 Gene Ontology gene sets.**

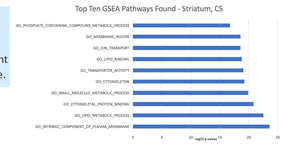
Step 6: GSEA - Hippocampus. The genes that were significant through age in the hippocampus **participate in pathways that are directly related to the brain and synaptic function.**
Nearly all pathways found were related to synaptic activity.



Step 6: GSEA - Frontal Cortex. The genes that were significant through age in the frontal cortex were involved in the same pathways as the hippocampus, but did not exhibit the same pattern correlating development to disease . Some pathways were involved in synaptic function.



Step 6: GSEA - Striatum. The genes that were significant through age in the striatum played no part in its function.
These pathways did not overlap with movement pathways associated with Huntington's Disease.



Conclusion

The genes that changed the most in development were decreased in Alzheimer's, but I didn't find this in FTD or Huntington's. This leads to the following significant conclusions:

- **My hypothesis was disproved - not all neurodegenerative diseases exhibit deregulation of developmental genes**
- **Alzheimer's is different from other neurodegenerative diseases (if not unique) and shows a correlation between the neurodegenerative disease and hippocampus development**
- **Different structures of the brain must have fundamentally different patterns of development in order for these findings to be true.**

My findings show that in Alzheimer's (but not necessarily other neurodegenerative diseases), **the same genes important to the childhood and post-conception development of memory in the brain are affected and actually deregulated in Alzheimer's disease.**

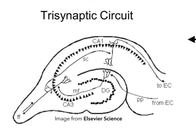
These findings are made even more significant, because other neurodegenerative diseases, specifically FTD and Huntington's **do not exhibit this same pattern**. This finding, although disproving my overall hypothesis, merits far more study. It not only sheds light on the development of Alzheimer's disease, but on that of other neurodegenerative diseases as well.

Discussion

There could be several possible reasons for differences between Alzheimers and FTD and Huntington's. One possibility:

Structural Differences

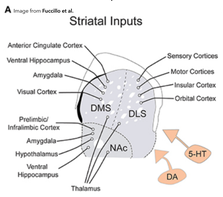
Trisynaptic Circuit

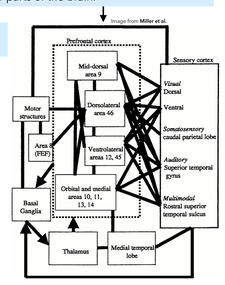


Alzheimer's Disease: The hippocampus has a simple, self-contained synaptic circuit consisting of three major connections

Frontotemporal Dementia: In contrast, the frontal cortex has a complex synaptic circuit consisting of several major connections to other parts of the brain.

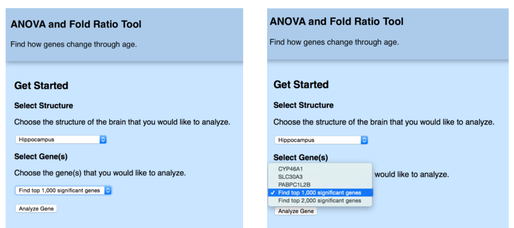
Huntington's Disease: The striatum makes very complex connections as well.





Future Work

- Create **API** to allow researchers to quickly and efficiently analyze genes in different structural regions of the brain. The API has a website as the front end and a Python backend. It uses MongoDB to store the current on-disk data and allow queries on the data. I am currently working on debugging the back end and plan on submitting this API to Allen Atlas



- Use API to analyze more neurodegenerative diseases - **Parkinson's Disease** and **Amyotrophic Lateral Sclerosis (ALS)** . Find out if all neurodegenerative diseases fit the same pattern as FTD and Huntington's
- Eventually contribute to a cure for these diseases!