Short-Chain Fatty Acids and the Impact on Dyslexia and ADHD Gene Expression
Gut Health and Your Brain: Empowering Through Diet

Why this Matters

Dyslexia Statistics
- ❑ According to the CDC, dyslexia affects 1 in 5 people
- ❑ 9.8% of children have been diagnosed with ADHD

Impact
- ❑ These disorders can significantly impact a person's self-esteem
- ❑ Current medication can lead to unintended consequences

Introduction

SCFAs and Parkinson's Disease:
- ❑ Recent research has found that SCFAs (Short-Chain Fatty Acids) have a positive effect in preventing and alleviating Parkinson's disease specifically through the gut-brain axis (see Figure 1)[2,3]

Role of SCFAs:
- ○ SCFAs are produced by gut bacteria during the fermentation of dietary fiber.
  - ■ SCFAs play a crucial role in:
    - Gut health
    - Immune function
    - Metabolism (see Figure 2)

Figure 1 *Gut-Brain Axis*

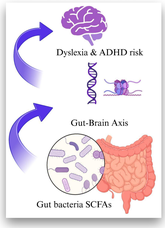


Figure 2 *SCFA process*

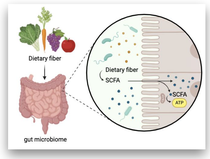
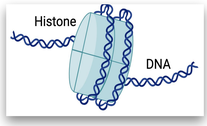


Figure 3 *Epigenetic effect*



Comorbidity
- ❑ Neurological disorders like dyslexia, Parkinson's, and ADHD often exhibit comorbidity and share genetic traits (pleiotropy)[1]

Epigenetics Overview:
- ❑ Epigenetics involves temporary changes in DNA gene expression through the chemical modification of histones, which are proteins around which DNA wraps.
- ❑ This process allows genes to be turned on and off.
- ❑ Epigenetic gene expression can be influenced by environmental factors such as diet and lifestyle (see Figure 3)

Visual Abstract

Purpose: To investigate SCFA's epigenetic effect on *RPI-1* expression

The Experimental Hypothesis: Increasing SCFAs concentration will cause *RPI-1* gene expression to decrease

The Null Hypothesis: No statistical significant differences in experimental and control groups

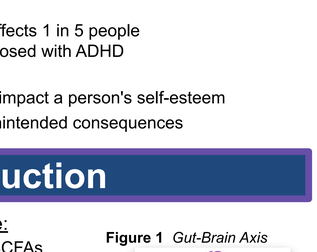
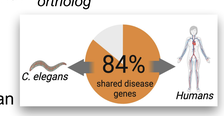


Figure 4 *Visual Abstract*

Experimental Design

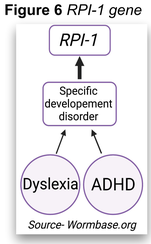
***C. elegans* as a Model Organism**:
- ❑ *C. elegans* share 84% of disease genes with humans, making it an effective model for studying genetic disorders (see Figure 5)

Figure 5 *C. elegans human ortholog*



***RPI-1* Insights:**
- ❑ The *RPI-1* gene in *C. elegans* is the human ortholog of the *DCDC2 (Doublecortin Domain Containing 2)* gene, which has been linked to dyslexia as well as ADHD sharing comorbidity (see Figure 6)

Figure 6 *RPI-1 gene*

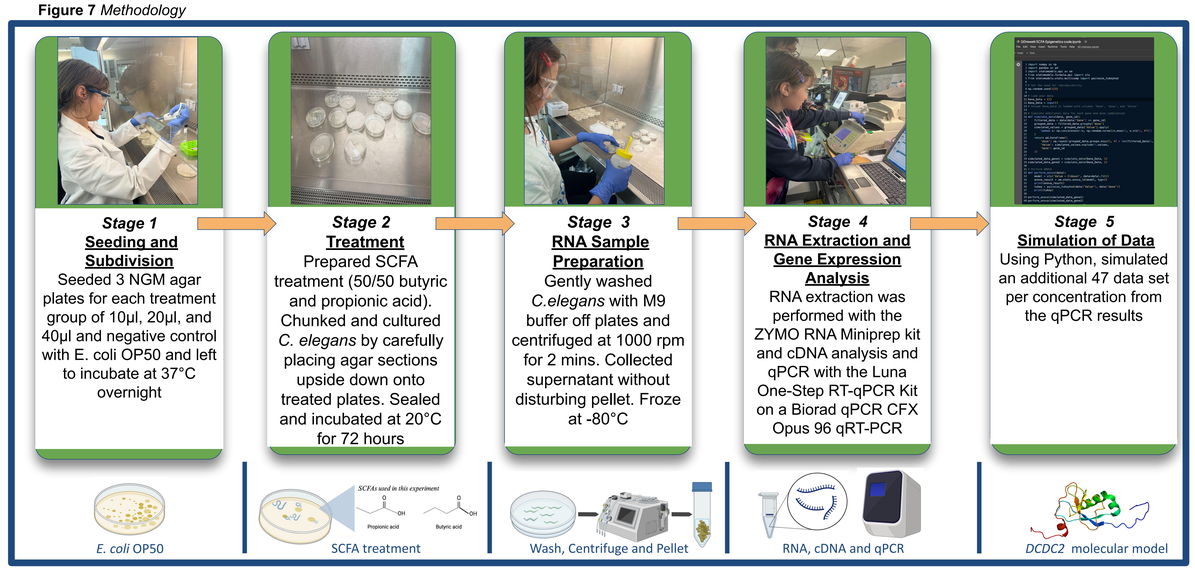


Experimental Variables:
- ❑ Dependent variable: Negative Control, 10µl, 20µl and 40µl concentrations of SCFA (50/50 butyric and propionic acid split)
- ❑ Independent variable: The gene expression of *RPI-1*
- ❑ Negative Control: Untreated *C. elegans*

Table 1. *Primer Sequence*

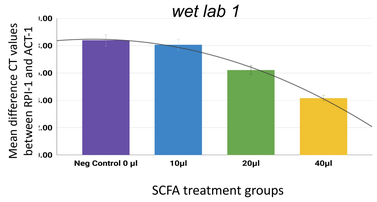
Gene	Primer Sequence
RPI-1	Forward: CTA GCA CGA TAT GAA TGA CTG Reverse: GGT AAT TTC AGC ATC TAA GC
ACT-1	Forward: ACG ACG AGT CCG GCC CAT CC Reverse: GAA AGC TGG TGG TGA CGA TGG TT

Figure 7 *Methodology*



Results, Statistical Analyses & Limitations

Figure 8 *Graph of difference in mean ct values of RPI-1 and ACT-1 wet lab 1*



Originally, the results did not show significance, however given the normality of the data and it's evenness, the observed declining pattern in *RPI-1* expression was deemed noteworthy

Graph of simulated qPCR results Ratio of amount of gene expression of simulated RPI-1 to ACT-1 per SCFA treatment

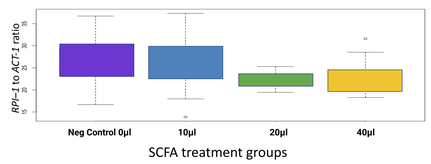
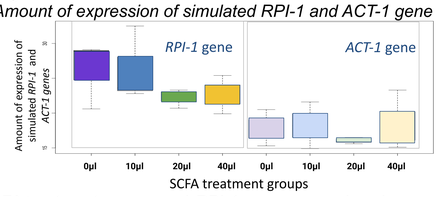


Figure 9b *Graphs of simulated qPCR results - Amount of expression of simulated RPI-1 and ACT-1 gene*



- ❑ SCFA treatment groups and *RPI-1* gene expression show a notable **observed pattern** (*see Figure 9a*). This implies that higher levels of SCFAs *may* lead to lower levels of *DCDC2* because it is the human ortholog of *RPI-1*
- ❑ The Tukey tests show significance at the 20µl SCFA treatment group level onwards at *p*-adjusted values of less than 0.05
- ❑ Figure 9b shows that the *RPI-1* gene was found to increase **without any *highly* significant increase in *ACT-1*** verifying that the treatment is not affecting other bodily mechanisms in a negative way

Results found to be Significant
Anova Test *p*-value: 1.494e-06 < 0.05 (alpha)
Tukey HSD Test for *RPI-1*:
Significant at 0-20µl,0-40µl,10-20µl,10-40µl
Shapiro-Wilk Normality Test *p*-value:
0.583 > 0.05 (normal)

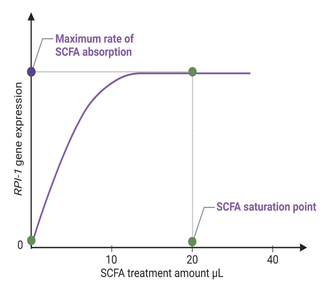
Statistical Analysis Outcome:
- ❑ **Null Hypothesis: Rejected**
- ❑ No observed differences can be attributed to chance alone
- ❑ **Experimental Hypothesis: Accepted**
- ❑ Indicates that an increase in SCFA concentration results in a decrease in RPI-1 gene expression related to dyslexia

Table 2.
TUKEY HSD p-value of RPI-1 CT groups 95% CL

CT	10-0	20-0	40-0	10-20	40-10	40-20
RPI-1	0.932	$1x10^{-8}$	$1.9x10^{-6}$	$1x10^{-9}$	$1x10^{-8}$	0.919
ACT-1	0.812	0.038	0.646	0.002	0.991	0.001

Significance of *ACT-1*:
- ❑ Notable at various points, but does not undermine the consistent, dose-dependent trend in *RPI-1* expression
- ❑ The robustness and consistency of *RPI-1* p-values across concentrations support this trend

Figure 10 *SCFA Saturation point*



Overlap Explanation:
- ❑ May be due to differing **dose sensitivity** between *ACT-1* and *RPI-1*
- ❑ Could also result from experimental handling and **SCFA saturation points**
- ❑ **No Impact at 0 to 10µl:** Indicates potential dose sensitivity or initial rejection of foreign substances.
- ❑ **20 to 40µl Plateau:** Suggests saturation where higher SCFA concentrations don't impact gene expression due to a limited number of SCFA receptors or a maximum rate for SCFA transport into cells

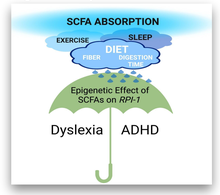
Future Directions and Challenges

My research showed that SCFAs are linked to RPI-1 gene expression in C. elegans. This suggests that SCFAs could influence dyslexia and ADHD in humans. This finding highlights how diet can affect gene expression related to these conditions.

Factors that aid SCFA absorption are:
- ❑ Foods that promote **faster colon transit time**,
- ❑ **Foods that promote less inflammation**
- ❑ **Exercise that boosts gut motility**
- ❑ **healthy sleep patterns**

These factors should be further researched for their potential in managing symptoms of dyslexia and related disorders

Figure 11 *Dyslexia Diet*



Limitations

- ❑ **Human Relevance:** Humans have more complex digestive systems
- ❑ **Lab Environment:** Controlled lab environment vs real life
- ❑ **Small Sample Size and Limited Budget:** 3 samples per group limits reliability
- ❑ Additional epigenetic influences and factors need to be explored such as sleep, exercise and climate

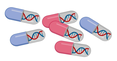
Future Work

- ❑ **Investigating SCFAs in Development:**
  Goal: Study how SCFAs (short-chain fatty acids) and gut bacteria affect the *RPI-1* gene in *C. elegans* through multiple generations
  Importance: This research could show how diet influences genes linked to dyslexia and ADHD early on
- ❑ **Expanding to Complex Organisms:**
  Goal: Conduct experiments with zebrafish, mice, and eventually humans
  Importance: Understanding SCFAs in various organisms may help find treatments for dyslexia and ADHD
- ❑ **Long-Term Studies on Lifestyle Factors:**
  Goal: Examine how diet, sleep, and exercise influence SCFA production and gene expression over time.
  Importance: Identifying key lifestyle habits could lead to effective dietary recommendations for managing dyslexia and ADHD symptoms
- ❑ **Collaborative Research Initiatives:**
  Goal: Partner with schools and dyslexia support groups for research that includes individuals' experiences
  Importance: This collaboration ensures that research addresses real challenges faced by people with dyslexia

Biotechnology Applications

- ❑ **Precision Nutrition or a 'Dyslexia Diet':**
  - ○ My research might lead to diets tailored to an individual's epigenetic makeup to prevent or manage dyslexia as well as ADHD
  - ○ I am currently developing a prototype app that spreads awareness about SCFAs and managing diet and sleep using CDC and FDA recommendations and findings from scientific papers

- ❑ **Epigenetic Drug Development:**
  - ○ This study may guide the creation of drugs using SCFAs to target neurological disorders



- ❑ **Diagnostic Biomarkers:**
  - ○ SCFA-based biomarkers could facilitate early detection, continuous monitoring, and personalized care for neurological disorders, benefiting both mothers and developing fetuses.

Key References

1. Clark, G. M., & Lum, J. A. G. (2017). Procedural learning in Parkinson's disease, specific language impairment, dyslexia, schizophrenia, developmental coordination disorder, and autism spectrum disorders: A second-order meta-analysis. Brain and cognition, 117, 41–48. https://doi.org/10.1016/j.bandc.2017.07.004
2. Cryan, J. F., O'Riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., Codagnone, M. G., Cussotto, S., Fulling, C., Golubeva, A. V., Guzzetta, K. E., Jaggar, M., Long-Smith, C. M., Lyte, J. M., Martin, J. A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., ... Dinan, T. G. (2019). The microbiota-gut-brain axis. *Physiological Reviews, 99*(4), 1877–2013. https://doi.org/10.1152/physrev.00018.2018
3. Hirayama, M., Nishiwaki, H., Hamaguchi, T., & Ohno, K. (2023). Gastrointestinal disorders in Parkinson's disease and other Lewy body diseases. *NPJ Parkinson's Disease, 9*. https://doi.org/10.1038/s41531-023-00511-2 **Paper bibliography contains full list*