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## Summary:

- Is there a statistically significant difference in gene expression of *AIF* in male and female *Drosophila melanogaster* (fruit fly) heads under controlled conditions?
- RNA from the heads of male and female fruit flies was extracted and analyzed using qRT-PCR.
- Results showed an 8.7-fold increase in *AIF* expression in male fruit flies when compared to females with  $p \geq 0.05$ .

## Abstract:

In this study we quantified the expression of apoptosis-inducing factor (*AIF*) gene in male and female fruit flies under controlled conditions. Apoptosis, or programmed cell death, is an important part of normal cell functioning. *AIF* contributes to the cell death pathway. Our results showed an 8.7 fold difference in male to female expression of *AIF*.

## Introduction

### Hypothesis:

There is no statistically significant difference in gene expression of *AIF* within male and female fruit fly heads under controlled conditions.

### Significance:

This project is a critical first step in understanding the physiological differences between male and female organisms. The findings of this project can help researchers in understanding the unique biological characteristics of men and women. Today, drugs are tested primarily on male organisms before being introduced as treatment options for humans. This means that these treatments may be better suited to treat males since no consideration is taken for how females may respond differently to medication or treatment. This project can help raise awareness so that more efficient medical solutions can be found that treat individuals based on their sex.

### Background:

#### Fruit Flies as a Model Organism:

We used fruit flies as our model organism because they are inexpensive and have a well characterized genome. They also have short lives and reproductive cycles, allowing for larger sample sizes. Many disease related genes in flies have homologs in humans, making flies a good model organism for the study of disease.<sup>1</sup> We used fruit fly heads because we wanted to study tissue specific gene expression, and brains have high metabolic activity.

#### Gene Function/Significance:

*AIF* (Apoptosis-inducing factor) facilitates an organized cell death, also known as apoptosis. Apoptosis is controlled cell death<sup>2</sup>.

## Methodology

### Fly culturing

Molasses food source; flies were maintained at 25°C



### Gender Differentiation

Differentiating between sexes to achieve gender specific results.



### Dissecting Heads

Isolating head with entomology forceps



### RNA Extraction

Adding a series of buffers and centrifuging to remove proteins, DNA, degraded RNA, cell debris, and excess salts.

- RNeasy Plus Mini Kit



### qRT-PCR

RNA is amplified and amplification is measured in real time to show the quantitative difference between male and female *AIF* RNA levels.

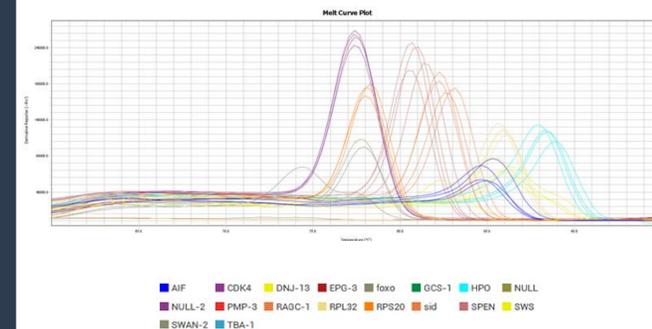
Primer sequences (5'-3'):

**Forward:** CCCCGTTGAAGAGTACGAGAC

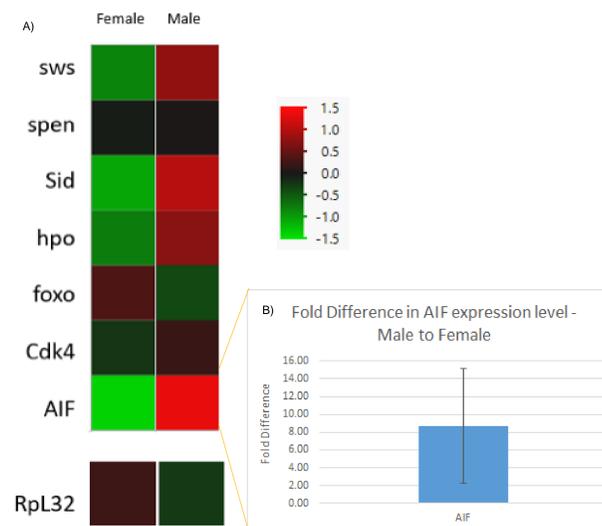
**Reverse:** AGCAAAGCCTTTAAAGAGTGGCTC

**Data Analysis:** Statistical significance was determined using unpaired t-test with  $p\text{-value} \leq 0.05$  and > 2-fold difference.

## Results



**Figure 1: Melt curve plot for qRT-PCR.** Dark blue peaks indicate melting temperature of *AIF* qRT-PCR products.



**Figure 2. Male and female gene expression differences.** A) *AIF* gene expression in fruit flies is normalized to housekeeping gene *Rpl32*<sup>3</sup>. A heatmap was subsequently produced using JMP Pro which indicates a higher gene expression with red and a lower relative gene expression with green. B) About 8.73 fold difference in the *AIF* gene expression between male flies and female flies  $p > 0.05$ .

## Conclusion

- There was a variation among the expression levels of *AIF*, which resulted in a  $p$  value > 0.05.
- There is possible upregulation of *AIF* in male flies. We observed an 8.7-fold difference between male and female *AIF* gene expression.
- We cannot draw the conclusion that increased *AIF* in males indicates increased apoptosis directly, as we have not measured the protein levels nor quantified apoptosis phenotype.
- This fold difference between males and females may be an important indicator of differences between the sexes, however, repeated experiments will be necessary.

### Study Limitations:

Individual differences were not quantified as 30 gender-specific fly heads were pooled for each biological sample.

### Future Experiments:

- Repeat in a more controlled environment and see if the results are replicable.
- Check if there is still a statistically significant difference in the expression under stressed conditions.
- Research the process of apoptosis in fruit flies to discover why there would be such a difference at baseline conditions.

## Bibliography



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