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Summary

- Are tumor suppressing mechanisms different between males and females?
- Quantified gene expression of the gene *l(3)mbt* in fruit fly brains via qRT-PCR.
- *l(3)mbt* showed 5.09-fold greater expression in males than females.

Abstract

Females and males have differences in behavior, brain gene expression, and response to stimuli. This experiment compares male and female *Drosophila melanogaster* (fruit fly) brains by quantifying *l(3)mbt* mRNA expression. Our results show a 5.09-fold higher expression of *l(3)mbt* mRNA in males compared to females, indicating a difference in tumor suppression between the sexes.

Introduction

Hypothesis: *l(3)mbt* gene will show higher levels of expression in males relative to female fruit flies.

Target gene:

- The *l(3)mbt* gene is a tumor suppressor involved in preventing cell proliferation in the optic lobes of the brain [1].
- Hereditary tumor syndromes are correlated with neurodevelopmental disorders such as autism [2].
- Neurodevelopmental disorders are characterized by impairments of growth and development of the brain and are more common in males [1].
- Less *l(3)mbt* mRNA expression results in less production of tumor suppressors and can increase the risk of getting a tumor.

Drosophila melanogaster (fruit flies):

- Share around 20,000 genes with humans [3].
- Share 75% of human disease related genes [3].
- Reproduce quickly, have a short life span of 2 weeks, and are inexpensive to maintain.

Materials and Methods

Separation of flies by gender

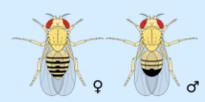


Figure 1. Females have a larger abdomen than males. Males have sex combs, and dark pointed genitalia [3]. Flies are raised at 23 °C on a molasses-based diet.

Separation of virgins



Figure 2. Photo of mature male, mature female, and virgin female flies (left to right).

Males and females are collected within 4 hours post-eclosion and fed 5% sucrose for 72 hours [4].

Fly brain dissection

Extracted male and female fly brains in PBS via microdissections and stored at -80°C

RNA extraction & DNase treatment

Fly brains were homogenized using silicone beads RNA was extracted using Trizol/chloroform method. Isopropanol and ethanol were used to concentrate RNA. Samples were DNase treated and stored at -80°C. RNA yield was determined via Nanodrop.

qRT-PCR

l(3)mbt primers (5'-3'):
Forward: CAGAGGAGTACGCAGTTGTATG
Reverse: CGGAGGTTCTTGGCAATTA

Performed qRT-PCR using iTAQ Universal SYBR Green Kit.

Data Analysis

$2^{-\Delta\Delta Ct}$ analysis was performed on the qRT-PCR data to determine the fold difference.

Figure 3. Flowchart of experiment progression.

Results

Sex	[RNA]	A260/A280
Male	97.6 ng/uL	1.97
Female	58.9 ng/uL	2.04

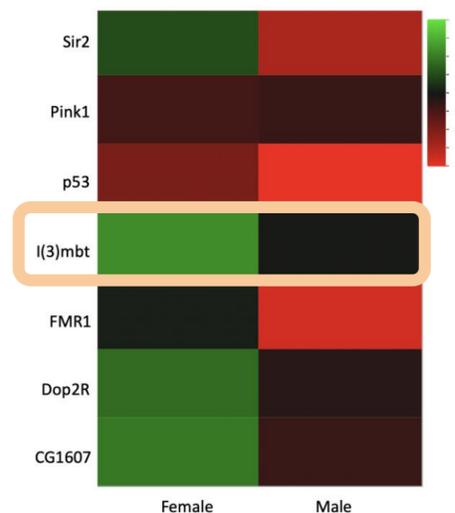
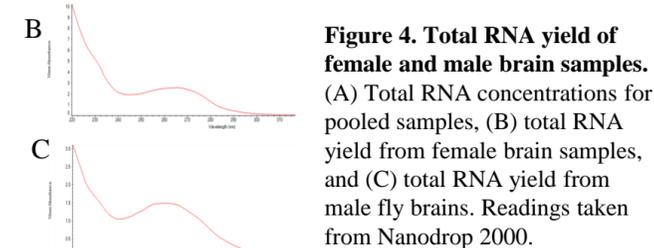


Figure 5. *l(3)mbt* mRNA expression is 5.09-fold higher in males relative to females. Red indicates a higher expression level and green indicates a lower expression level. All genes normalized to *gapdh1*. This figure was generated using JMP Pro 14.

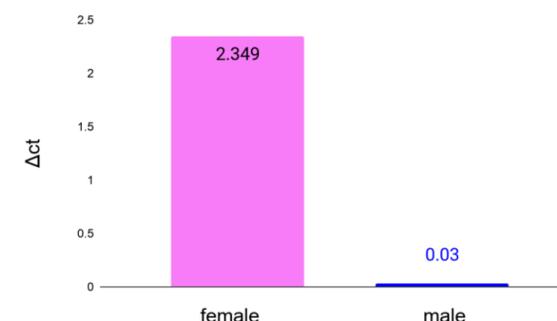


Figure 6. ΔCt values of *l(3)mbt* are higher in females than males. ΔCt value for female is 2.349 and 0.03 for males. A larger CT value indicates less starting RNA. mRNA levels normalized to *gapdh1*. The data was obtained through the Quantstudio 3 system.

Discussion & Conclusion

l(3)mbt exhibits sexually dimorphic mRNA expression in fruit flies.

- *l(3)mbt* mRNA expression is 5.09-fold higher in males relative of females, supporting the hypothesis.
- This suggests post-eclosion males have higher tumor-suppressing capabilities than females.
- The human ortholog of *l(3)mbt* is *h-l(3)mbt* [5]. Differential expression of *h-l(3)mbt* could indicate differences in tumor suppression between the sexes.

This study further supports a need for sex-specific studies and establishes baseline sexual dimorphism of *l(3)mbt*.

Study Limitations

- Flies were only studied at the post-eclosion life stage.
- Individual variation was not measured
- Study lacks statistical analysis.

Future Directions

- Study *l(3)mbt* mRNA expression at different life stages.
- Explore individual fruit fly variation of *l(3)mbt* expression.
- Repeat experiment with a larger sample size.

References

