

Fmr1 is expressed 3.714-fold higher in male *Drosophila melanogaster*



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Summary

- Are the markers related to cognition different between male and female brains?
- RNA was extracted from brains of virgin male and female fruit flies and *Fmr1* mRNA expression levels were measured through qRT-PCR.
- Males demonstrated a 3.714-fold higher *Fmr1* expression.

Abstract

Fmr1 is also known as the Fragile X mental retardation gene and regulates mRNA that code for FMRP proteins related to cognitive functions. This experiment investigated the difference in *Fmr1* mRNA expression between male and female *Drosophila melanogaster* (fruit fly) brains. Analysis of qRT-PCR data shows male fruit flies express more *Fmr1* mRNA by 3.714-fold, indicating sex-specific regulation of cognition.

Introduction

Hypothesis: As Fragile X syndrome affects males more than females in humans, *Fmr1* will be expressed more in male relative to female fruit flies.

Drosophila melanogaster:

- 75% of disease-causing genes in humans are found in fruit flies [1].
- Fruit flies have short life cycles, have many offspring, are easily differentiable by sex, and are easily maintained [1]



Figure 1: Photo of an adult male (top) and a virgin female (bottom) fruit fly.

Target gene:

- *Fmr1* codes for the FMRP protein that controls mental development [2,3].
- When it is not expressed, the cognitive disability Fragile X Syndrome occurs [2,3].
- Fragile X Syndrome is more prevalent in males [3].

Methodology

Fly Keeping/Separation of Sex:
Flies were raised on a molasses-based diet; ≤ 4 hours post-eclosion, female and male virgin flies are separated and collected

Female Control:
Once collected, females are fed 5% sucrose for 72 hours

Male Control:
Once collected, males are fed 5% sucrose for 72 hours

Sample Collection:
Flies were anesthetized, stored at -80°C , and fly heads were dissected to isolate the brain

RNA Extraction:
Samples were:

- Pooled (100 fruit fly brains per sex)
- Homogenized in TriZol and chloroform
- Precipitated in isopropanol
- Washed with 75% ethanol
- Resuspended in nuclease free water
- Treated with DNase
- Stored at -80°C

qRT-PCR:
Fmr1 Primer Sequences:

- Forward Primer: 5'-CGTCAGGAGAAGATGGAGATG-3'
- Reverse Primer: 5'-GTGGTTGTGGTGCTGATAGT-3'

Data Analysis:
Calculated fold difference ($2^{-\Delta\Delta\text{Ct}}$) from qRT-PCR data

Figure 2: Flowchart of experimental procedure.

Results

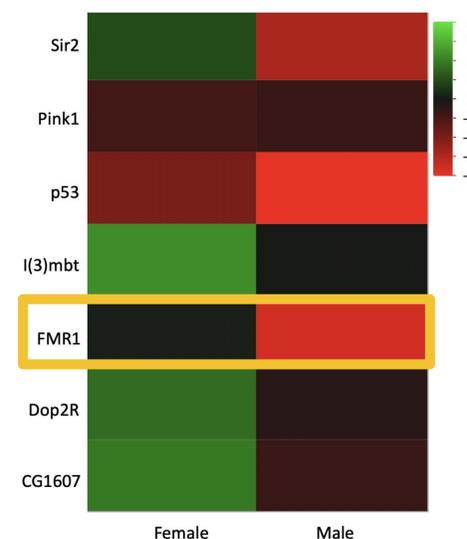
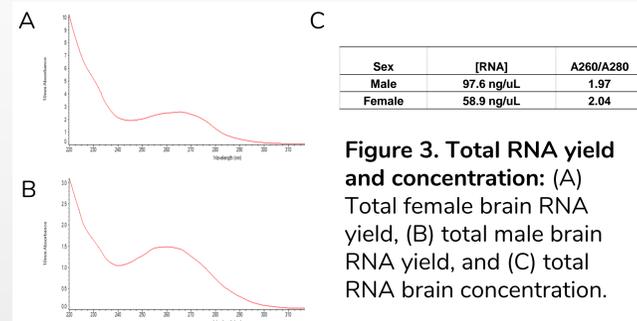
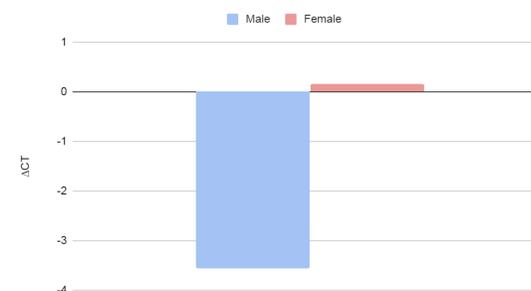


Figure 5: Lower ΔCt values for male relative to female fruit flies: ΔCt value for male 0.159 and ΔCt value -3.555 for females. Normalized to *gapdh1*. Data obtained through the QuantStudio 3 system.



Discussions & Conclusion

Fmr1 mRNA expression is sexually dimorphic.

- Under control conditions, male flies exhibit 3.714-fold higher levels of *Fmr1* mRNA relative to female fruit flies.
- This result agrees with the hypothesis that males express more *Fmr1* mRNA than females.
- Due to the lack of a second X chromosome in human males, higher expression of *Fmr1* may be necessary to prevent Fragile X syndrome [2,3].

The sexual dimorphism demonstrated by *Fmr1* suggests males and females in research should be studied separately and provides a baseline difference in *Fmr1* expression future studies can refer to.

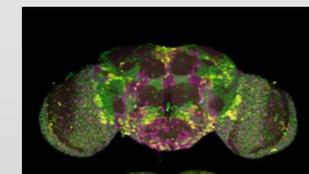


Figure 6. Fluorescent microscope image of fruit fly brain [4]. The fruit fly brain is easy to isolate and accounts for about 14% of the fly body weight.

Study Limitations & Future Directions

- Only one technical replicate was used for this study. More samples should be analyzed to establish statistical significance.
- Fly behavior was not observed. A behavioral assay based on *Fmr1* expression and stressors could help understanding phenotypic effects of altered *Fmr1*.
- Flies are evolutionarily distant from humans. Replicate the study onto a different model organism to see if the effects are conserved.
- Only post-eclosion flies were studied. Flies from different life-stages should be studied to see if this expression is maintained life-long.

References

