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

**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, and are easily differentiable by sex, and are easily maintained [1].

**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; 48 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'-CGTCAGGAAGATGAGATG-3'
  - Reverse Primer: 5'-GTGGTTGTGGTCTGATAGT-3'

**Data Analysis:**
- Calculated fold difference (2-ΔΔCt) from qRT-PCR data.

**Results**

- **Figure 3. Total RNA yield and concentration:**
  - (A) Total female brain RNA yield, (B) total male brain RNA yield, and (C) total RNA brain concentration.

- **Figure 4. FMR1 mRNA expression is 3.174-fold greater in males relative to females:**
  - Gene expression future studies can refer to.

- **Figure 5. Lower ΔCT values for male relative to female:**
  - Normalized to gapdh1. Heatmap was generated using JMP Pro 14.

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

**Discussion & 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 will 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.

**References**

- 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.