

Expression of *Pink1* is higher in female fruit flies relative to males by 1.8-fold



Nguyen T.¹, Gutierrez R.¹, Martinez A.¹, Vu J.^{2,3}, Engelhart D.^{2,3}

¹Clairemont High School, ²Boz Life Science Research and Teaching Institute, ³UCSD Extended Studies

Summary

- Do females and males respond to oxidative stress differently?
- Male and female fruit fly brains were dissected, and RNA was extracted for qRT-PCR analysis of *Pink1*.
- Females have 1.8-fold higher expression of *Pink1* than males.

Abstract

The *Pink1* gene protects cells from stress-induced mitochondrial dysfunction. This experiment established a baseline for the differences in *Pink1* mRNA expression between male and female *Drosophila melanogaster* (fruit fly) brains. The *Pink1* gene was targeted with qRT-PCR and data was analyzed based on CT values showing females had higher *Pink1* mRNA expression by 1.8-fold. This result highlights similar expression patterns between males and females.

Introduction

Hypothesis: Since Parkinson's disease is more prevalent in males, and *Pink1* protects against oxidative stress, *Pink1* will be expressed more in female relative to male fruit flies.

Target gene:

- *Pink1* contains instructions for the PTEN induced putative kinase 1 protein shown to protect cells from oxidative stress-induced mitochondrial dysfunction [1].
- *Pink1* is expressed in the brain and is linked to the neurological disorder Parkinson's disease [2].

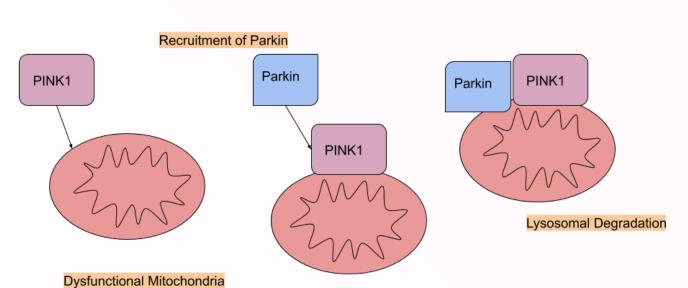
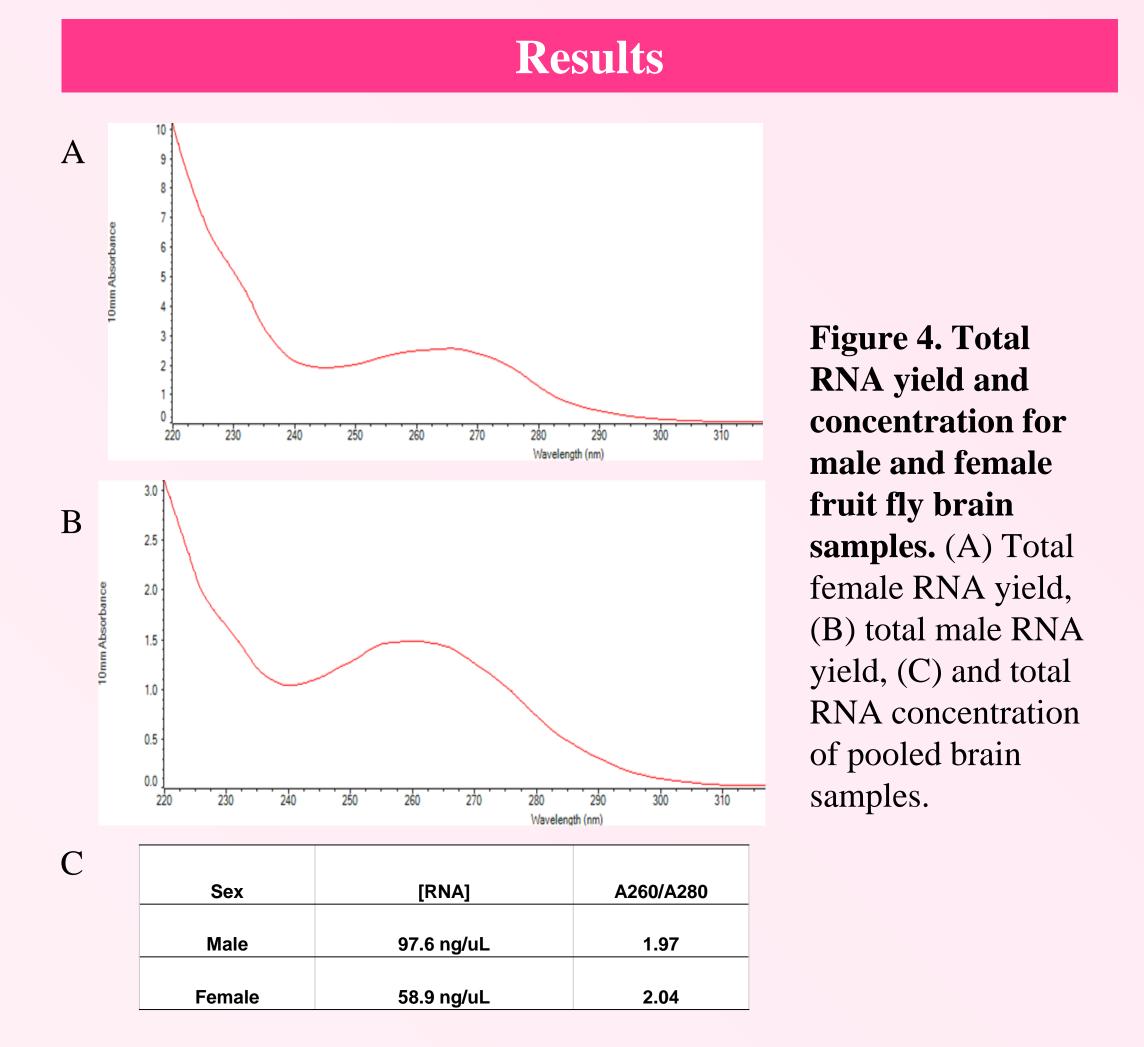


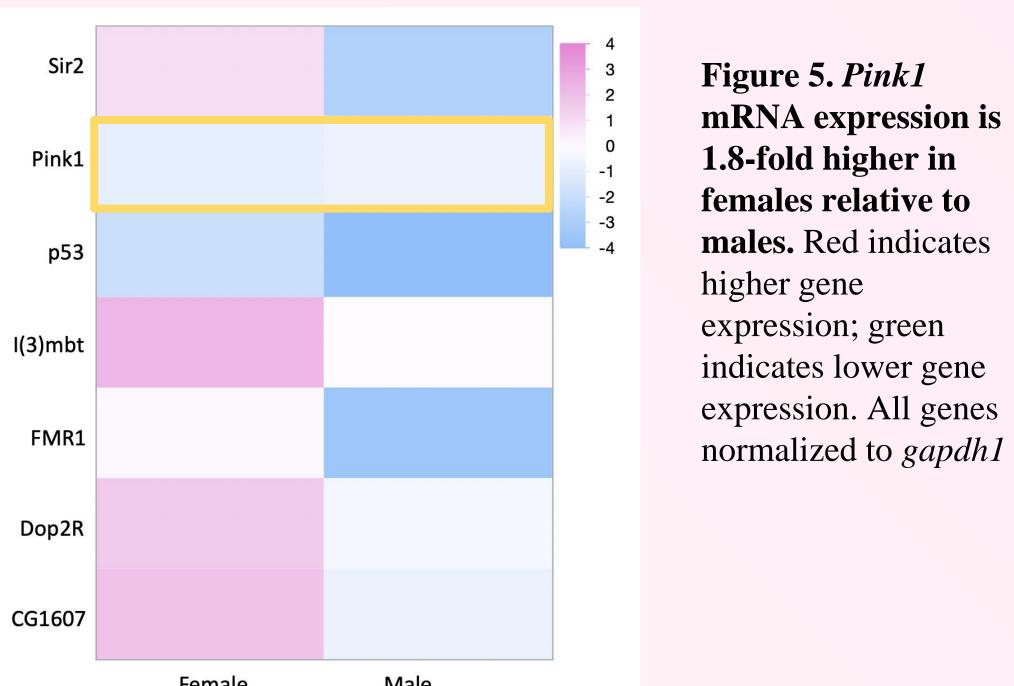
Figure 1. *Pink1* Pathway:
The protein coded by *Pink1*recognizes and attaches to
the dysfunctional
mitochondria. It then recruits
the Parkin protein to attach
to the mitochondria. This
causes the dysfunctional
mitochondria to undergo cell
lysis [1].

Model organism:

• *Drosophila melanogaster* (fruit flies) share about 75% of disease-related genes with humans, are inexpensive to maintain, have many offspring, and reproduce quickly [3].

Methodology Fly Keeping and Separation by Sex Flies were raised on a molassesbased food. Male and female virgin flies were separated ≤4h posteclosion. Female-control Male-control Female flies were fed a Male flies were fed a 5% sucrose solution 5% sucrose solution for 72h for 72h Figure 2. Male fruit fly: Male fruit flies can be identified by the presence of sex combs and morphological differences on the abdomen [4, 5]. Sample Collection: Flies were frozen $\leq 76h$ post-eclosion at -80°C and brains are extracted under a microscope. RNA Extraction: Isolated fruit fly brains were pooled by sex and RNA extracted using Trizol and chloroform. Using isopropanol, RNA was centrifuged down into a small pellet. Using 70% ethanol the RNA was washed. It was then treated with DNase in nuclease free water and stored at -80°C. qRT - PCRPink1 gene was targeted using the following primers Forward Primer 5' CGACGATTTCGCCTGTAGT 3' Reverse Primer 5' CTCCTGTGCCTGCAACTATTC 3' Data Analysis • *Pink1* was normalized to *gapdh1* • Afterwards $\Delta\Delta$ CT was found by subtracting the Δ CT of males from Δ CT of females Fold difference in gene expression was calculated using 2^{- ΔΔCT} Figure 3. Flowchart of experimental design.





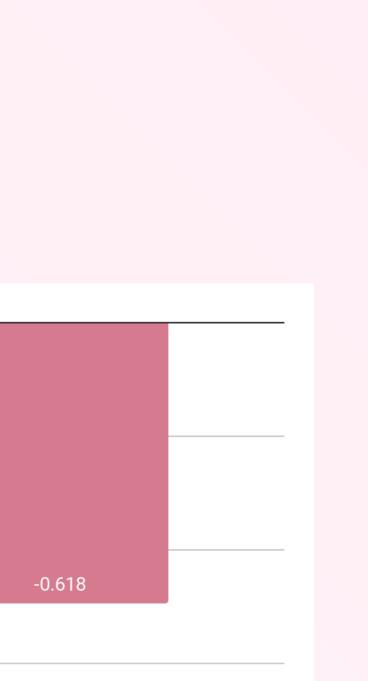


Figure 6. ΔCT values are lower for female fruit flies relative to males. The difference in cycle threshold, after being normalized with *gapdh1*, is greater in male fruit flies with -0.618 than female fruit flies with -0.864, resulting in a 1.8-fold higher *Pink1* mRNA expression in female fruit flies.

Female

Discussion/Conclusion

Pink1 does not exhibits sexually dimorphic mRNA expression in fruit flies.

- In control conditions, female *Drosophila melanogaster* have 1.8-fold higher gene expression of the *Pink1* gene.
- Although these results agree with the hypothesis, the slight 1.8-fold upregulation of *Pink1* is not significant enough to indicate sexual dimorphism in *Pink1*.
- Males are affected by Parkinson's more often than females [6]. Non-sex-specific expression of *Pink1* could indicate the protective effects are not sexually dimorphic.

This study showing differences in *Pink1* in fruit flies establishes baselines for future studies of *Pink1* expression under stressors.

Study Limitations

- Flies were studied only post-eclosion.
- Brains were pooled no distinction between individuals.
- Study lacks statistical analysis.
- Study was only performed on mRNA level.

Future Directions

- The experiment could be performed in higher order organisms.
- Analyze individual genetic expression within the sexes.
- In future experiments, gene expression data could be collected from flies at other life stages.
- Protein expression of *Pink1* could be analyzed.

Bibliography

