Expression of *Pink1* in male and female *D. melanogaster* is unaffected by GenX treatment

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Summary

- Do *Drosophila melanogaster* (fruit flies) express different amounts of *Pink1* after exposure to GenX?
- Do female and male fruit flies express different amounts of *Pink1* under control conditions?
- Gene expression was quantified using qRT-PCR
- There were no significant changes in expression of *Pink1* across any of the conditions.

Abstract

GenX is an unregulated chemical with no conclusive evidence about its toxicity. Male and female organisms have sexually dimorphic gene expression and may respond differently to stressors. In this study, we exposed fruit flies to GenX and measured the expression of the gene *Pink1* in both males and females and compared the baseline expression of the *Pink1* in both sexes. At baseline, females downregulated *Pink1* 1.21-fold compared to males (p-value = 0.955). After exposure to GenX, *Pink1* was upregulated in males by 11.43-fold (p-value = 0.494) and downregulated in females by 34.84-fold (p-value = 0.162). We conclude that there were no significant changes in expression of *Pink1* across any of the conditions.

Introduction

Hypothesis:
Exposure to GenX will cause downregulation of *Pink1* expression in *D. melanogaster*.
Pink1 will be expressed more at control conditions in males than females.

- **Gen X**
  an unregulated, human-made chemical from the family of PFAS chemical compounds used in commercial products such as packaging, non stick pan coatings, and firefighting foam.
- ***Drosophila melanogaster***
  model organism chosen for genetic similarity with humans, short lifespan with clear developmental stages, and clear differences between males and females.
  - ➢ *Drosophila melanogaster* share 75% of disease genes with humans, and have many common organs and organ systems with humans.
- **Target Gene: *Pink 1***
  Pink 1 is thought to help protect the mitochondria from malfunctioning during periods of cellular stress like unusually high energy demands

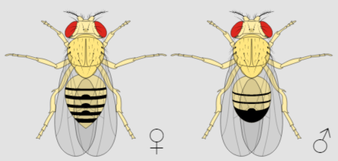

Figure 1: Diagram of male and female *Drosophila melanogaster*. This diagram shows distinct differences between the two sexes.

Methodology

Fly maintenence and collection:
Flies are grown at 22-25°C and separated by sex ≤4h post-eclosion.

Control condition: Flies were fed cornmeal based food for 7 days

Experimental condition: Flies were fed cornmeal based food infused with 1000 mg/kg of GenX for 7 days.

Sample collection:
Flies were sacrificed by freezing at 80°C and brains were dissected, with 100 brains pooled per sample. RNA was extracted using the RNeasy kit (Qiagen) and quality was verified using a nanodrop.

qRT-PCR
Pink1 primers:
Forward primer 5'->3': CAATGTGCTGCGGACAACG
Reverse primer 5'->3': AGATTCCACTGCTGCTGGTG

Data Analysis and Bioinformatics:

- *Pink1* was normalized to *Actin*
- Fold difference in gene expression was calculated using $2^{-\Delta\Delta CT}$
- ΔΔCT was found for the different conditions that were compared by taking the ΔCT difference between control female and control males, as well as between experimental condition and control condition for both males and females.
- A Student's t test was performed using ΔCT values.
- A p-value of less than 0.05 was considered statistically significant.
- STRING protein interactions and gene homology analysis was conducted to better understand the role of *Pink1*

Figure 2. Experimental outline

Results

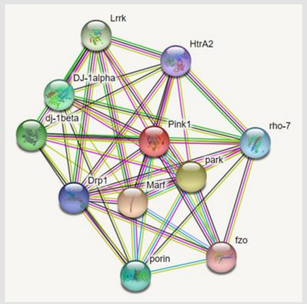

Figure 3. STRING model showing protein interactions. Most of the related proteins are involved in mitochondrial processes or oxidative stress responses. This figure was generated in STRING (https://string-db.org/).

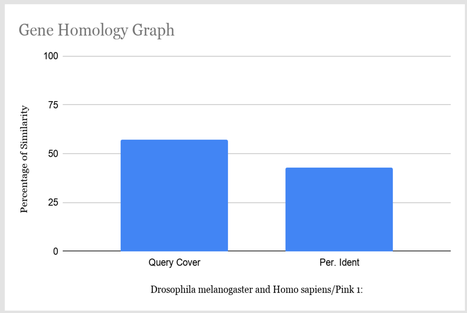

Figure 4. Bar graph showing the gene homology between *Homo sapiens* and *Drosophila melanogaster* in relation to pink1 gene. The percent identity of *Pink1* in *Homo sapiens* and *Drosophila melanogaster* is about 43%, this means that the gene sequence of *Pink1* is 43% identical in both species. The query cover is 57%, meaning that the target sequence spans 57% of the query sequence.

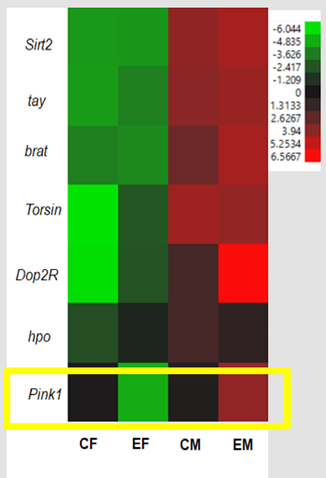

Figure 5. Heatmap showing different levels of gene expression in response to GenX. *Pink1* is highlighted in yellow. The legend shows that high expression level is indicated by red, and low expression level is indicated by green. The expression of *Pink1* in GenX-exposed females was downregulated 34.8-fold (p-value = 0.162), and upregulated 11.43-fold in GenX-exposed males (p-value = 0.494). Female fruit flies express *Pink1* 1.21-fold less than male fruit flies under control conditions (p-value = 0.955).

Discussion & Conclusion

- **Control condition**
  - Slightly lower expression (0.827-fold) comparing female to male *D. melanogaster* (p = 0.954)
- **Female fly exposure**
  - Decreased but not statistically significant *Pink1* expression (0.0287-fold) in females exposed to GenX (Figure 5)
  - Decreased expression of *Pink1* could affect synaptic function, which is linked to Parkinson's disease if it is maintained for a long period of time
  - The difference is not statistically significant due to the t-test resulting in a p-value of 0.162
- **Male fly exposure**
  - Increased gene expression in males exposed to GenX chemicals (Figure 5)
  - Increased *Pink1* might be caused by oxidative stress or an increased demand for mitophagy
  - These results are not statistically significant due to the results of a t-test which resulted in a p-value of 0.494
- **No statistically significant difference in *Pink1* expression level was observed, although the relatively large p-values could be caused by large variations across experimental replicates**

Limitations and Future directions

- Flies were exposed to one concentration of GenX. Further testing should include a range of concentrations.
- Flies were exposed to GenX at adulthood. Future testing should include exposure at different life stages, such as larvae or embryos.
- Each condition had 2-3 data points. Future experiments should include a greater number of replicates in order to account for potential outliers skewing the results. With more samples, we can compare variability in the data.
- Our research only tests the effect on flies of GenX exposure through food. Future experiments can look at the effects of using other methods of exposure to GenX.

Sources