

Expression of *Pink1* in fruit fly brains does not have a significant change when exposed to GenX, a potential toxicant



Gonzalez A.¹, Owusu-Hienno V.², Essein D.³, Hayford M.⁴, Stewart C.^{5,6}

¹Castle Park High School, ²The international School of Beaverton, ³Ghana National College, ⁴Aggrey Memorial Senior High, ⁵Boz Life Science Research and Teaching Institute, ⁶UC San Diego Extension

Summary

- Male and female fruit flies were exposed to 1000 mg/kg-day GenX
- Differences in gene expression in the brain between the sexes and conditions were measured using qRT-PCR
- We report no statistically significant changes in the expression of *Pink1*.

Abstract

Research on the effect of toxicants is necessary to determine safe levels of exposure. However, very often research is conducted solely on males and is not generalizable to females due to physiological differences between males and females. Our research project uses *Drosophila melanogaster* (fruit flies) to assess the difference in gene expression of *Pink1* between female and male fruit flies and fruit flies that have been exposed to a potential toxicant GenX using qRT-PCR. We found no significant changes in gene expression of *Pink1* between any of the conditions.

Introduction

Hypothesis

- Pink1* is expressed more in male fruit flies than female fruit flies.
- Pink1* is upregulated in fruit flies after exposure to GenX as a response to the stress.

Fruit fly

- Short generation times
- About 75% of known human disease genes have a recognizable match in the genetic code of fruit flies¹
- 40% of fly protein sequences have mammalian analogues¹
- Easy to distinguish between male and female flies



Figure 1. Differences between female and male fruit flies. Female flies are generally larger, and have light, pointed abdomen, while male flies have dark, rounded abdomen.

GenX

- Replacement chemical for PFOA, a toxic PFAS chemical
- Used in nonstick items like pans, rain jackets, and food packaging
- Not regulated by the EPA
- No conclusive evidence for safety level and toxicity

Pink1

- PTEN-induced putative kinase 1, a protein coding gene
- Pink1* is expressed at greater levels in adult male flies²
- Pink1* is the ortholog of human *PINK1*, a gene mutated in autosomal recessive Parkinson's disease
- PINK1* encodes a mitochondrially targeted Ser-Thr kinase
 - Involved in the degradation of a dysfunctional mitochondria and the proper functioning of components in the electron transport chain.

Methodology

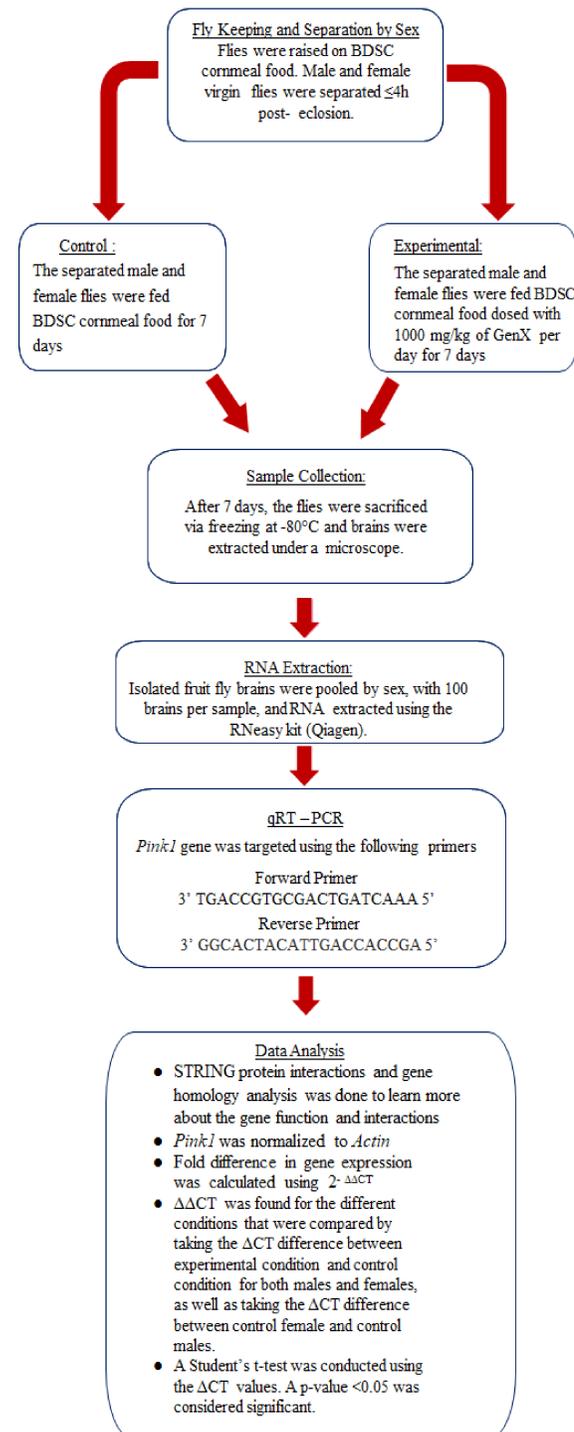


Figure 2 Flowchart of experimental design.

Results

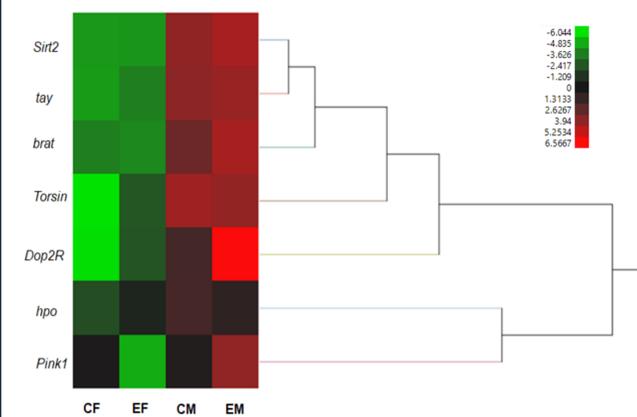


Figure 3. Heatmap showing gene expression for 7 genes under various conditions. CF indicates control female, EF indicates experimental female, CM indicated control male, and EM indicates experimental male. Green indicates relatively less expression, and red indicates relatively more expression. This heatmap was generated in JMP Pro 14 using Δ CT values normalized to *actin*, a housekeeping gene. *Pink1* expression fold changes for the following conditions were observed: there is a fold change of 0.0287 when comparing experimental female to control female (p-value= 0.162), 11.434 when comparing experimental male to control male (p-value=0.494), and 0.827 when comparing control female to control male (p-value=0.954).

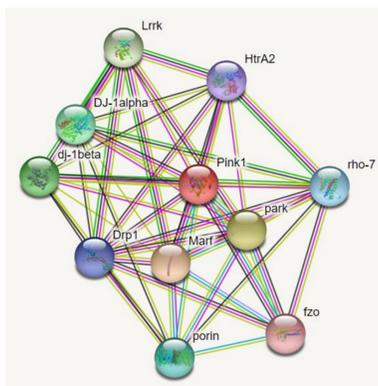


Figure 4. Proteins that interact with *Pink1*. Most of the related proteins are involved in mitochondrial processes or oxidative stress responses. This figure was generated in STRING (<https://string-db.org/>).

Query Cover and Per. Indent of PINK 1 Gene in *Danio rerio*, *Homo Sapiens*, and *Caenorhabditis elegans*.

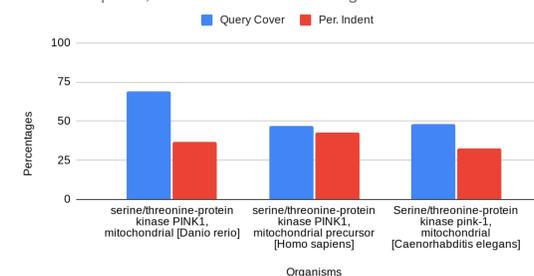


Figure 5. Conservation of the *D. melanogaster* *Pink1* gene among *D. rerio*, *H. sapiens*, and *C. elegans*. Query cover represents the extent of similarities in gene length, while Per. Indent indicates the extent of similarities in the gene sequence. This figure was generated using information from NCBI homologue database and Protein Blast.

Discussion

- Pink1* is a protein thought to protect cells from stress-induced mitochondrial damage.³
 - Most of the proteins associated with *Pink1* on the STRING protein diagram are involved in oxidative stress response (Figure 4).
- We report the following fold differences in *Pink1* expression levels across experimental groups, none of which are statistically significant:
 - 0.827-fold comparing control female flies to control male flies (p = 0.954)
 - 0.0287-fold comparing GenX-exposed female flies to control female flies (p = 0.162)
 - 11.434-fold comparing GenX-exposed male flies to control male flies (p = 0.494)
- Unexpectedly, we did not find a significant difference between *Pink1* expression levels in control female and male flies.
 - This suggests that *Pink1* does not have a sexually dimorphic gene expression, which does not agree with previously reported data.²
- Based on our data, we conclude that *Pink1* is not significantly expressed differently between female and male flies, and exposure to GenX does not significantly affect *Pink1* expression level.
 - Due to the high fold-change differences, we suspect that the relatively high p-values were caused by large variations across experimental replicates.
 - This could be verified with more repeats of this experiment.

Limitations and Future Directions

- Only one time point was used to compare the effects of GenX on male and female fruit flies.
- In the future, different lengths of exposure to GenX can be used.
- We measured gene expression in pooled fly brains, which might have masked individual response differences.
- Individual fruit fly brains to better understand individual variation in response to GenX
- This experiment can be repeated with more replicates to obtain more consistent data with smaller variations.
- The effects of GenX exposure can be compared to other model organisms

References

- Pandey, U. B., & Nichols, C. D. (2011). Human disease models in *Drosophila melanogaster* and the role of the fly in therapeutic drug discovery. *Pharmacological reviews*, 63(2), 411–436. <https://doi.org/10.1124/pr.110.003293>
- FlyBase Gene Report Dmel_Pink1. <https://flybase.org/reports/FBgn0029891>
- PINK1 PTEN induced kinase 1 [Homo sapiens (human)] - Gene - NCBI. <https://www.ncbi.nlm.nih.gov/gene/65018>