

Torsin is significantly upregulated in Male fruit fly brains compared to Female fruit fly brains

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Summary

- Does exposing *Drosophila melanogaster* (fruit flies) to GenX have any effects on the expression of *Torsin*?
- How does the expression of *Torsin* differ between male and female fruit flies?
- Gene expression of *Torsin* in male and female fruit flies with and without exposure to GenX was measured using qRT-PCR
- *Torsin* was downregulated 1048.03 fold from female to male controls

Abstract

Scientific studies, particularly drug response studies, are often conducted using male organisms. This ignores the sexually dimorphic gene expression between males and females. Here we exposed fruit flies to GenX, a chemical with relatively unknown health effects, and quantified the difference in expression of *Torsin* in female and male fruit flies, and the baseline expression of female vs male control flies using qRT-PCR. Female and male GenX-exposed flies showed a 11.78-fold upregulation (p-value = 0.0549) and 1.23-fold upregulation (p-value = 0.841) when compared to control, respectively, and female control flies showed a 1408.5-fold downregulation when compared to male control flies (p-value = 0.0129). We conclude that females express *Torsin* significantly less than male fruit flies.

Introduction

Hypothesis

Exposure to GenX will increase expression of *Torsin* in male fruit fly brains due to them having a much stronger reaction to dopamine compared to female brains.

Drosophila melanogaster (fruit fly)

- ◆ Shares 75% of human disease-related genes⁷
- ◆ Easy to take care of and exposure to various treatments
- ◆ Sexually dimorphic⁴
- ◆ Commonly used model organism



Figure 1. Adult fruit fly.

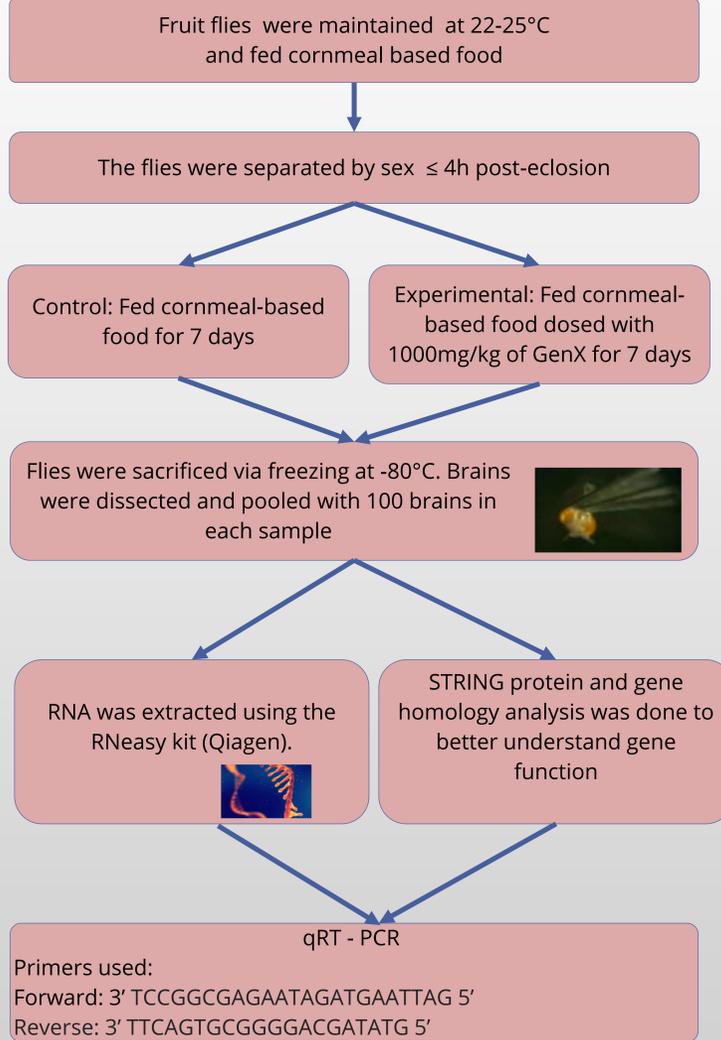
Torsin³

- ◆ Conserved in both humans and fruit flies
- ◆ Involved in ATP binding and ATPase activity⁹
- ◆ Regulates dopamine metabolic process, neuron cellular homeostasis, and positive regulation of cellular growth

Gen-X

- ◆ Used to make non-stick coatings, firefighting foam, food packaging¹⁵
- ◆ Manmade chemical and created to be a replacement for PFAS/PFOA chemicals^{13,14}
- ◆ Has been found in both rain and groundwater⁶

Materials and Methodology



Data Analysis

- *Torsin* expression was normalized to *Actin*.
- Fold difference in gene expression was calculated using $2^{-\Delta\Delta CT}$
- $\Delta\Delta CT$ was calculated for the pairs of 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.
- Student's t test was performed using ΔCT values. If the p-value is < 0.05 , the data is considered significant.

Figure 2. Experimental overview

Results

Gene Homology

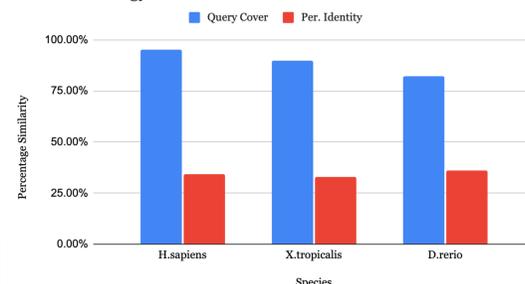


Figure 3. Gene Homology for *torsin* genes and their coded proteins in three other species. This figure shows how much the homologous genes overlap with one another/compare to one another lengthwise (query cover), and how much the homologous genetic sequences match that of the target gene (percentage identity). *H.sapiens* - query cover is 95%, P. Iden. is 34.35%. *X.tropicalis* - query cover is 90%, P. Iden. is 35.92%. *D.rerio* - query cover is 82%, P. Iden. is 33.01% (10, 11, 12)

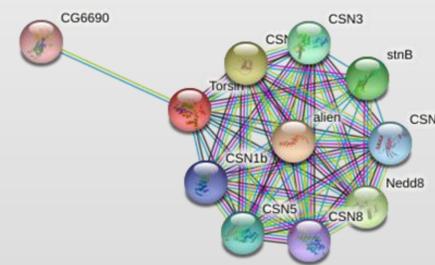


Figure 4. Predicted protein interactions from the STRING Protein database. The connections represent when proteins are predicted to be functionally connected with one another. The database suggests that the group of proteins connected to Torsin may play a role in the following biological processes: protein deneddylation, male germ-line cyst encapsulation, female germ-line stem cell population maintenance(2)

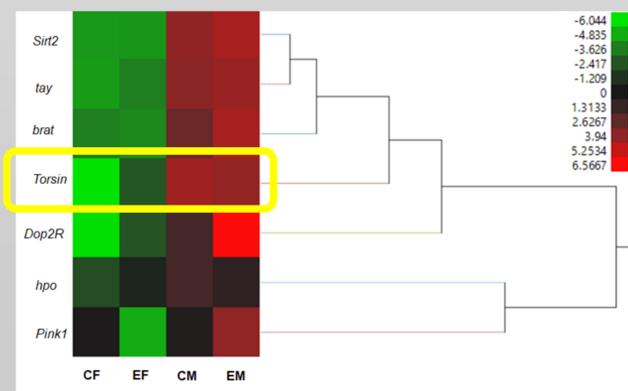


Figure 5. Heat map of the ΔCt values of the experiment. Shows all four conditions by column (Control Female, Experimental Female, Control Male, Experimental Male, from left to right) for all the target genes. *Torsin* is circled in yellow. *Torsin* was upregulated 11.78 fold in the EF condition from the CF condition, downregulated 1.23 fold in the EM from the CM condition, and downregulated 1408.03 fold in the CF condition from the CM condition. However, using the t-test, the data was only found to be significant ($p < 0.05$) in the comparison between males and female control groups. p -values in each of the comparisons: 0.0129 (CF to CM), 0.0549 (EF to CF), 0.8405 (EM to CM).

Discussion and Conclusion

- Female fruit flies have a statistically significant lower *Torsin* expression level than male flies at baseline ($p < 0.05$).
- There is no significant effect on the gene expression of *Torsin* due to the GenX exposure through fly food.
 - o For female exposure vs. control groups, $p = 0.0549$
 - o For males exposure vs. control groups, $p = 0.8405$
- *Torsin* has a part in ATP binding and ATPase activity which lets us conclude that
 - o Female fruit flies tend to utilize ATP less compared to male fruit flies
 - o GenX exposure did not affect ATP utilization in fruit flies
- *Torsin* relates to proteins like stnB and CSN3 (fig. 3) which are related to many cellular metabolic and developmental processes.
 - o Because *Torsin* is related to ATP utilization, difference in *Torsin* expression level could have implications on cellular energy management and affect the other cellular functions as mentioned above.

Study Limitations and Future Directions

- GenX exposures were only conducted on adult life stages
 - Future experiments can begin exposing the fruit flies at earlier developmental stages
- The effects of GenX exposures was only studied on the fruit fly brain
 - RNA can be extracted from other organs to study changes in gene expression
- Flies were only exposed to GenX for 7 days and other time points were not measured
 - Flies can be exposed to GenX for varying lengths of time

Sources Used

