

# The dopamine-regulatory gene *torsin* is sexually dimorphic in male and female *Drosophila melanogaster*.

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## Summary

- ❖ *D. Melanogaster* were exposed to the potentially toxic chemical GenX
- ❖ Expression of *Torsin* was quantified using qRT-PCR
- ❖ *Torsin* is significantly downregulated in female compared to male *D. melanogaster* at control conditions

## Abstract

The health effects of GenX, a man-made chemical used in the production and fabrication of paint, food-packaging, and non-stick coatings, are widely unknown. Additionally, male and female organisms may have sexually dimorphic gene expression. To analyze the effects of GenX, we exposed *Drosophila melanogaster* to the chemical and analyzed the expression of *Torsin*, a gene involved in regulating dopamine levels, using qRT-PCR. We found *Torsin* to be upregulated 11.78-fold in females (p=0.055) and downregulated 1.23-fold in males (p=0.841) after exposure to GenX. Females express *Torsin* 1408.5-fold less than males at control conditions (p=0.0129). We conclude that males express significantly more *Torsin* than females at baseline.

## Introduction

**Hypothesis** - GenX exposure decreases the expression of *Torsin* in *D. melanogaster*. Male *D. melanogaster* will express more *Torsin* at baseline compared to females.

**GenX** - A chemical used in the fabrication of paint, food packaging, and non-stick coatings. Adverse health effects are unknown.

***D. melanogaster*** - has approximately 75% of disease-causing genes homologous to those of humans, is easy to distinguish between sexes, and is a well-established model organism

***Torsin*** - utilized in the biological process of biogenesis, localization and development. *Torsin's* molecular function is ATP binding. *TOR1A* is the human ortholog.

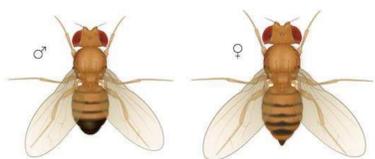


Figure 1. Adult male and female *D. Melanogaster*. Males have a solid black tail tip, whereas females have a striped tail end.

## Methodology

**Fly Maintenance:** Flies were raised on BDSC cornmeal food. Male and female virgin flies were separated  $\leq 4$  h post-eclosion.

**Treatments:** Control flies were fed BDSC cornmeal food for 7 days and GenX-exposed flies were fed BDSC cornmeal food dosed with 1000 mg/kg per day for 7 days

**Sample collection:** After 7 days, fly brains were pooled by sex, with 100 brains per sample and frozen at  $-80^{\circ}\text{C}$ . RNA was extracted using the RNeasy kit (Qiagen) and stored at  $-80^{\circ}\text{C}$

**qRT-PCR:** *Torsin* was targeted by the following primers

Forward primer:  
3' TCCGGCGAGAATAGATGAATTAG 5'

Reverse Primer:  
3' TTCAGTGCAGGGGACGATATG 5'

### Data Analysis

- STRING protein interactions and gene homology analysis was done to learn more about the gene function of *Torsin*.
- *Torsin* was normalized to *Actin*.
- Fold difference in gene expression was calculated using  $2^{-\Delta\Delta\text{CT}}$
- $\Delta\Delta\text{CT}$  was found for the different conditions that were compared by taking the  $\Delta\text{CT}$  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 conducted using the  $\Delta\text{CT}$  values, where a p-value of less than 0.05 was considered significant.

Figure 2. Experimental design

## Results

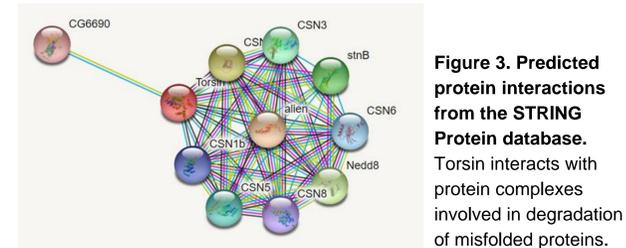


Figure 3. Predicted protein interactions from the STRING Protein database. *Torsin* interacts with protein complexes involved in degradation of misfolded proteins.

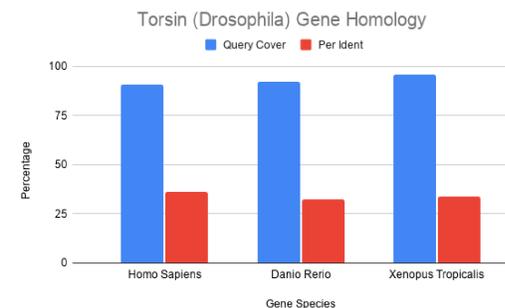


Figure 4. Gene homology for *Torsin* coded proteins in three other species. *H.sapiens* - query cover is 95%, P. Iden. is 34.35%. *X.tropicalis* - query cover is 90%, P. Iden. is 33.01%. *D.rerio* - query cover is 82%, P. Iden. is 35.92% (10, 11, 12).

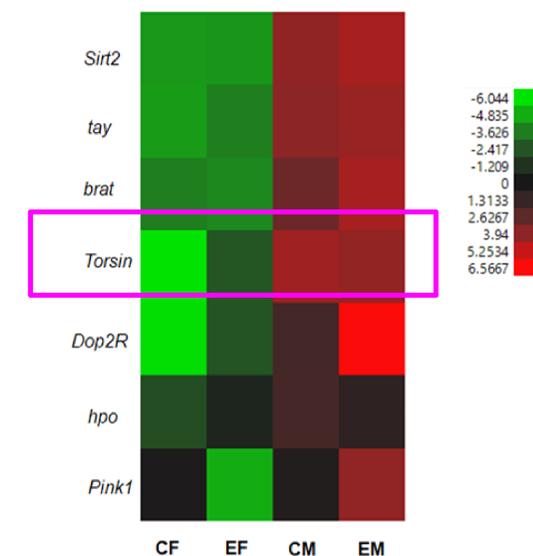


Figure 5: Heatmap showing expression levels of genes in Control and Experimental Males and Females tested using qRT-PCR. Red indicates a high expression level and green indicates a low expression level. CM indicates control males, CF indicates control females, EF indicates experimental females, EM indicates experimental males. *Torsin* is circled in pink. *Torsin* was upregulated 11.78 fold in the EF condition from the CF condition (p-value = 0.0549), downregulated 1.23 fold in the EM from the CM condition (p-value= 0.8405), and downregulated 1408.03 fold in the CF condition from the CM condition (p-value= 0.0129).

## Discussion & Conclusion

- *Torsin* is involved in ATP binding and transport of proteins from the nucleus to the cytoplasm
- *Torsin* gene expression is sexually dimorphic with a 1408 fold difference between the female and male control flies (p-value= 0.0129).
- GenX did not significantly impact the expression of *Torsin* gene in *D. melanogaster*, p-values > 0.05.
  - CF vs. EF: p-value is 0.0549
  - CM vs. EM: p-value is 0.8405
- Overall, this suggests that male flies utilize more ATP than female flies and potentially have a greater energy expenditure.
- GenX treatment did not have a significant effect on protein transport out of the nucleus or ATP use in the experimental flies.
- The effect of GenX on humans may correlate to that on fruit flies due to genetic similarity.
  - Query cover of 91% and Percent Identity of 36.31% for *TOR1A*, the ortholog of *Torsin* in humans (figure 4).
  - Human *TOR1A* expression might not be affected by GenX at an exposure level comparative to 1000 mg/kg in fruit flies.

## Limitations/Future Directions

- Individual physical variation among *D. melanogaster* was not accounted for when pooled for analysis. In the future, individual brains can be analyzed for gene expression.
- Only one GenX concentration was tested. In further experiments, there should be variation in concentrations, to study the effects on gene expression due to varying concentrations of GenX.
- *D. melanogaster* was exposed to GenX only in its adult stage. In future experiments, *D. melanogaster* can be exposed to GenX starting from earlier life stages to understand the effects on development.

## Sources

"Gene Ontology and GO Annotations." QuickGO. EMBL-EBI. 2020. Web. November 2020  
 "Gene Report: DmelTorsin." FlyBase. FlyBase. 14 October 2020. Web. November 2020  
 "Torsin [Drosophila melanogaster (fruit fly)]." NCBI. National Center for Biotechnology Information. 21 October 2020. Web. November 2020.  
 "Torsin Protein (Drosophila melanogaster)." STRING interaction network. STRING. 2020. Web. November 2020.  
 Grillet, M., Gonzalez, B. D., Sicart, A., Pottler, M., Cascalho, A., Billon, K., ... & Verstreken, P. (2016). Torsins are essential regulators of cellular lipid metabolism. *Developmental cell*, 38(3), 235-247.  
 Wakabayashi-Ito, N., Doherty, O. M., Moriyama, H., Brakefield, X. O., Gusella, J. F., O'Donnell, J. M., & Ito, N. (2011). Dtorsin, the *Drosophila* ortholog of the early-onset dystonia *TOR1A* (*DYT1*), plays a novel role in dopamine metabolism. *PLoS one*, 6(10), e26183.