

A tumor suppressor gene, *brat*, has sexually dimorphic gene expression in male and female *D. melanogaster*

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Summary

- Drosophila melanogaster* (fruit flies) were exposed to chemical GenX.
- We investigated changes in gene expression of *brat*, a tumor suppressor gene using qRT-PCR.
- brat* is downregulated 111-fold in female fruit flies relative to male fruit flies.

Abstract

GenX is a chemical using in nonstick coatings that may adversely affect organisms. We exposed fruit flies to GenX and quantified the difference in expression of *brat* between female and male flies for both non-exposed and GenX-exposed conditions using qRT-PCR. Female and male GenX-exposed flies showed a 1.2-fold downregulation (p-value=0.896) and 2.855-fold upregulation (p-value=0.405) when compared to control, respectively, and female control flies showed a 111-fold downregulation when compared to male control flies (p-value=0.035). We conclude that females express *brat* significantly less than male fruit flies.

Introduction

D. melanogaster

- Established model organism
- Sexual dimorphism, low cost, short lifespan, and shared evolutionary history with humans
- Drosophila* genome is 60% similar to human genome.¹

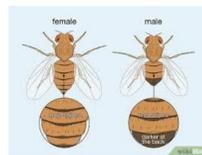


Figure 1. Adult male and female flies.

GenX

- Chemical used in nonstick coatings
- No conclusive evidence for toxicity or safety level

brat

- Tumor suppressor gene. Encodes proteins that regulate differentiation and growth.

Hypothesis

- Exposing fruit flies to GenX in their food source reduces expression of the *brat* gene.
- Male fruit flies express more *brat* than females.

Methodology

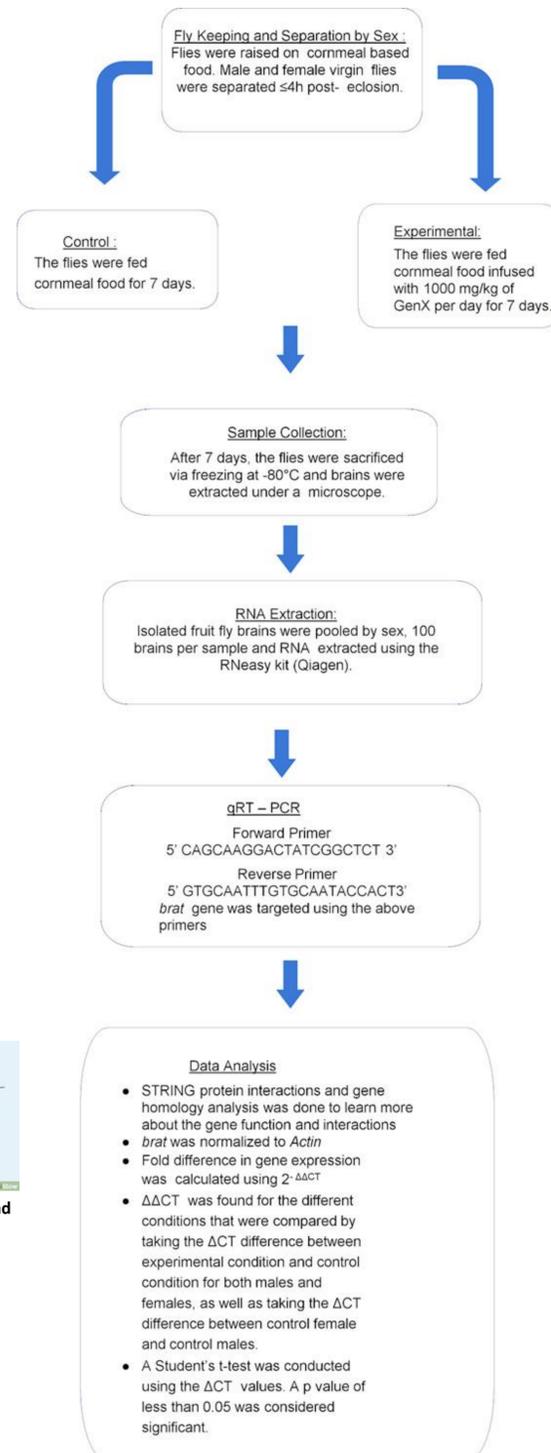


Figure 1. Experimental Design

Results

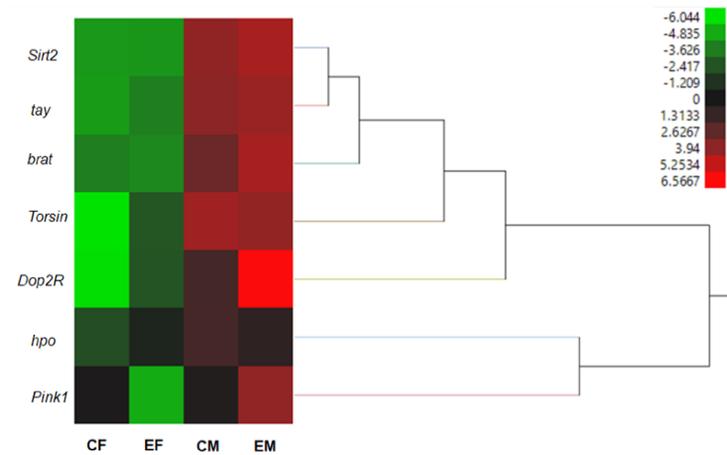
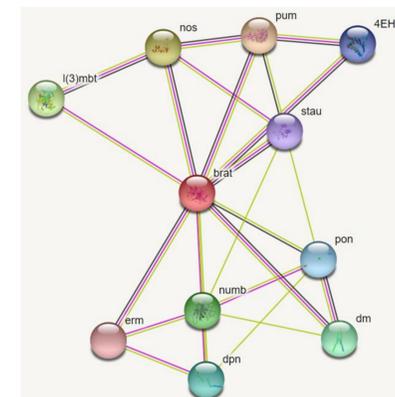


Figure 2. Hierarchical clustering of 7 genes under various conditions. Genes are clustered based on similarity in response between sexes and with or without exposure to GenX. Gene expression upregulation is marked by red, and downregulation is marked by green. *brat* was downregulated 1.2-fold when comparing experimental female to control female (p-value=0.896), upregulated 2.855-fold when comparing experimental male to control female (p-value=0.405), and downregulated 111-fold when comparing control female to control male (p-value=0.035). This heatmap was generated in JMP Pro 14 using ΔCt values normalized to a housekeeping gene, *actin*. CF indicates control female, EF indicates experimental female, CM indicated control male, and EM indicates experimental male.

Figure 3. STRING diagram showing proteins that interact with *brat*. Proteins connected to *brat* are involved in cell cycle regulation. This figure was generated in STRING (<https://string-db.org/>).



Discussion

- brat* is statistically significantly expressed less in female fruit flies compared to male ones (111-fold, $p < 0.05$).
- In humans, the ortholog of *brat*, *TRIM3*, is a tumor suppressor.²
 - If the expression pattern of *TRIM3* in human is similar to that of *brat* in flies, higher expression of *TRIM3* in males could indicate their higher demand of tumor suppression in brains.
- GenX does not significantly alter *brat* expression level in fruit flies (Figure 2).
 - Based on this result, we infer it is likely GenX did not induce response to brain tumor in fruit flies.

Limitations and Future directions

- Limitations:
 - Flies brains were pooled - variation among individual flies not considered.
 - D. melanogaster* only treated with one dosage of GenX for one time point
 - Flies do not have the same biological processes as humans
- Future directions:
 - Use brains from individual flies for analysis
 - Varied amounts of GenX exposure
 - Longer periods of exposure
 - Different 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>
- Chen, G., Kong, J., Tucker-Burden, C., Anand, M., Rong, Y., Rahman, F., ... & Brat, D. J. (2014). Human *Brat* ortholog *TRIM3* is a tumor suppressor that regulates asymmetric cell division in glioblastoma. *Cancer research*, 74(16), 4536–4548.