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Summary

- Are the markers of autism expressed differently in males and females?
- Investigated sexual differences in the mRNA expression of *tay*, a gene similar to *AUTS2* linked to autism in humans when mutated.
- *tay* was expressed 71.4-fold higher in males relative to female fruit flies.

Abstract

AUTS2 is a gene in humans that encodes a protein in developing brain regions (cerebellum, frontal cortex, hippocampus) implicated in autism neuropathology. *AUTS2* is similar to *tay* in *Drosophila melanogaster* (fruit fly). In this study, RNA was extracted and *tay* mRNA expression was measured by qRT-PCR for males and females to determine if *tay* expression is sexually dimorphic. The results show that *tay* is expressed higher in males by 71.4-fold, highlighting sex-specific gene expression associated with incidence of autism.

Introduction

Hypothesis:

- *tay* gene expression will be higher in female fruit flies relative to males.

Drosophila melanogaster (fruit flies):

- Highly conserved genome, sharing 75% of human disease-related genes.
- Short life-span.
- Quick reproduction time.
- Observable developmental stages and sex differences.¹



Figure 1. *D. melanogaster* at adult lifestage. Female fruit fly (left) and male fruit fly (right). Females have larger, pointed abdomens, males have darker abdomens and sex combs on their forelimbs.

Target gene:

- Encodes for protein LD2260p, present in all cell types².
- Mutations can affect 26 organs, including the brain and the wings of fruit flies².
- Human *AUTS2* mutations are correlated to autism^{3,4}.
 - Human males are more commonly diagnosed with autism than females

Methodology

Fly Collection

Flies were anesthetized with CO₂ and virgin flies were collected ≤4 hours post-eclosion

Determination of Fly Sex

Female Male

Male and female flies were identified and separated under a microscope

Isolation of Fly Brains

Dissected 100 male and 100 female heads, extracted fly brains.

RNA Extraction

RNA was extracted with TriZol/chloroform and then treated with DNase to remove DNA in RNA pellet.

qRT-PCR:

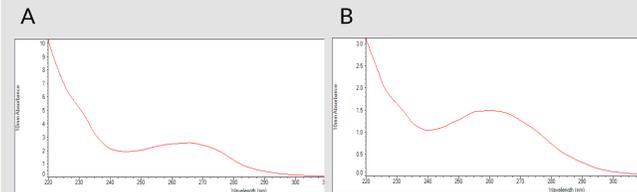
tay primers
 Forward (5'-3'): CGGACAGCGATCCAAGTAA
 Reverse (5'-3'): GTCGTCGTTCCACCAGAAATA

Performed qRT-PCR with iTaq Universal SYBR® Green Kit

Data Quantification:

Cycle Thresholds for *tay* were normalized to *gapdh1*. Fold differences ($2^{-\Delta\Delta CT}$) between sexes were calculated

Results



Sex	[RNA]	A260/A280
Male	97.6	1.97
Female	58.9	2.04

Figure 2. Total RNA yield and concentration for female and male fruit fly brain samples: (A) Total female RNA yield, (B) total male RNA yield, and (C) total RNA concentration of brain samples.

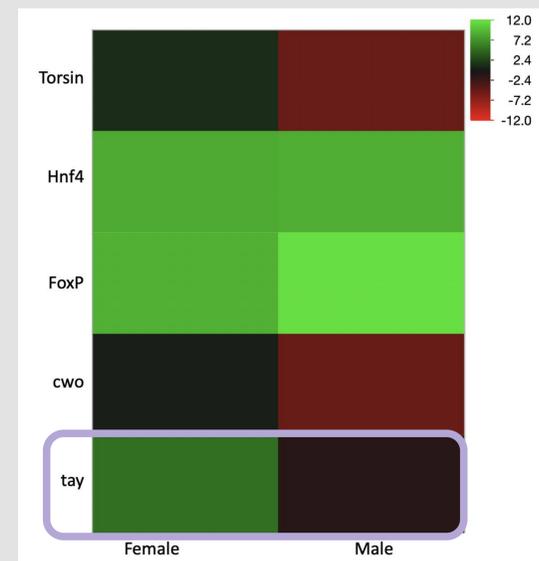


Figure 3. *tay* gene expression is 71.4-fold higher in males relative to female fruit flies: *tay* (highlighted) is shown to have a lower gene expression in females. Green indicates lower expression of the gene and red indicates a higher expression of the gene. Normalized to *gapdh1*.

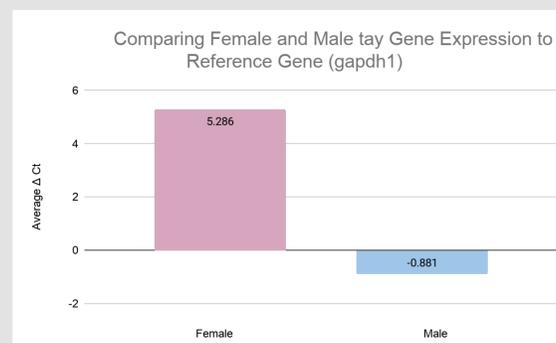


Figure 4. Normalized ΔCT of *tay* mRNA expression is lower in males relative to female fruit flies: ΔCT value for female flies is 5.286 and -0.881 for males. A lower ΔCT mRNA expression correlates to a higher mRNA expression level. *tay* normalized to reference gene *gapdh1*.

Discussion/Conclusion

tay exhibits sexually dimorphic mRNA expression.

- Results indicate *tay* is expressed 71.4-fold higher in male relative to female fruit flies, disagreeing with the hypothesis.
- Higher expression levels of *tay* in male *Drosophila* may indicate a more pronounced effect of any mutations.
- Mutations of the human ortholog *AUTS2* are linked to autism^{3,4}.
- Another autism-related gene, *CG1607*, is also expressed higher in male *Drosophila*.
- Sexual dimorphism of *tay* and *CG1607* supports previous research showing higher incidence of autism in males.

Quantifying sex-specific expression of *tay* in fruit flies establishes baselines for future studies on sexual dimorphism in *tay* in fruit flies under stressors.

Study Limitations

- Only one technical replicate; statistical significance could not be determined.
- Samples were pooled
- Fruit flies were only studied at one life-stage (post-eclosion)

Future Directions

- Study the gene expression of *tay* across different life stages in *D. melanogaster*.
- Study the gene expression of *tay* in a higher order organism.
- Investigate rate of mutations for *tay* in fruit flies and correlate to phenotypes.
- Quantify baseline *tay* protein levels

References

