Gender and head-specific gene expression study reveals that *hpo** & *sws* genes are respectively 3x & 4x upregulated in males compared to females understandard conditions

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Research Background

Neurodegenerative diseases, such as ALS, caused by loss of motor neurons affect men disproportionately compared to women[1]. While the exact cause of ALS is unknown, it is not attributed to sex hormones. Recent studies show gene expression for several non-sex related genes varies significantly based upon gender[2]. It is well established that the highly conserved Hippo signaling pathway regulates organ growth, cell proliferation, and apoptosis. It is also shown that the inhibition of the Hippo signaling pathway causes tumorigenesis[3]. However, hyper-activation of Hippo signaling pathway can lead to increased apoptosis, likely contributing to diseases affected by increased cell death like ALS, Arrhythmogenic Cardiomyopathy, and Diabetes. The Hippo gene (*hpo*) is responsible for the production of the Hippo protein kinase which is the key mediator for the Hippo signaling pathway. In contrast, Alzheimer's disease is more likely to affect women than men. 1 in 6 women are affected by Alzheimer's disease in addition to 1 in 11 men[4]. The *sws* gene controls neuronal and glial degeneration as well as apoptotic cell death[5]. Hyper-regulation of the *sws* gene results in a decreased chance of these degenerations and cell death. Alzheimer's disease as well as a low gene expression of the *sws* gene are both characterized by neuronal degeneration.

Our hypothesis: **"There is variation in the expression of the *hpo* and *sws* genes between males and females under normal conditions."**

Understanding gender differences of gene expression of the *hpo* and *sws* will allow us to better develop drugs for diseases affected by dysregulation of the cell apoptosis or cell proliferation pathways which disproportionately affect a particular gender.

Research Overview

- We used *Drosophila melanogaster* to study the gene expression of the *hpo* and *sws* genes. *Drosophila melanogaster* is an ideal organism as the *hpo* signaling pathway (hpo/SAV/WATS) is highly conserved across species and corresponds to the MST1/2/SAV1/LATS pathway in mammals[4].
- Flies were sorted by gender. 30 fly-heads of each gender, male and female, were used to average the gene expression. The samples were lysed, filtered and washed to isolate the RNA.
- Primers were designed to amplify mRNA transcribed from the *hpo* and *sws* genes and qRT-PCR was performed.
- The gene expression levels were normalized by comparing the Ct number against the housekeeping gene *RpL32* [6]. This in turn is used to determine the ratio of male to female gene expression levels.

Methodology

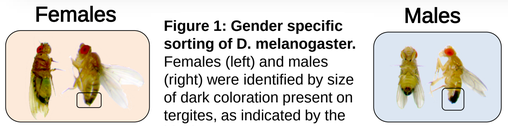

Figure 1: Gender specific sorting of D. melanogaster. Females (left) and males (right) were identified by size of dark coloration present on tergites, as indicated by the black square.

Step 1: Prepare Drosophila
- Used stock wild type Drosophila
- Sorted males vs. females
- Prepared 2 batches of male heads (MA & MB)
- Prepared 2 batches of female heads (FA & FC)
- Ground male and female heads in preparation for RNA extraction

Step 2: RNA Extraction
- RNeasy Mini Kit is used for RNA extraction
- Used Buffer RLT to lysate sample
- Used gDNA Eliminator spin column to remove DNA
- Removed excess carbohydrates, proteins, and fatty acids using buffer RWL
- Removed excess salts using buffer RPE
- Eluted RNA using RNase-free water

Step 3: qRT-PCR
- Used 25 ul of Go Taq Green Master Mix (DNTPs, Reverse Transcriptase, Sybr Green, etc)
- Added Forward Primer (2.5 ul), Reverse Primer (2.5 ul) to RNA extract
- Prepared positive and negative control vials along with housekeeping vials for qRT-PCR
- Used QuantStudio 3 to perform qRT-PCR

Step 4: Data Analysis
- Compared gene expression of each male relative to each other female ($2^{-\Delta\Delta CT}$)
- A p-value of < 0.05 using an unpaired t-test and 2-fold difference in gene expression was considered statistically significant.

Figure 2: Research Methodology

Gene Name	*hpo*
RefSeq (cDNA)	NM_001274163.1
Primer Set – Sequence 5'->3' (generated by PrimerBlast)	Forward: GAGCAAGGTGTGGATGAGGG
	Reverse: ATAGTGCCCAAGTTCGACTCCA

Table 1: mRNA primer sequence for *hpo*. Primers were designed with Primer-BLAST from NCBI.

Gene Name	*sws*
RefSeq (cDNA)	NM_167140.2
Primer Set – Sequence 5'->3' (generated by PrimerBlast)	Forward: TCACCCTCACCACCGACATA
	Reverse: TTATGCATCACATCGGCTGGC

Table 2: mRNA primer sequence for *sws*. Primers were designed with Primer-BLAST from NCBI.

Results

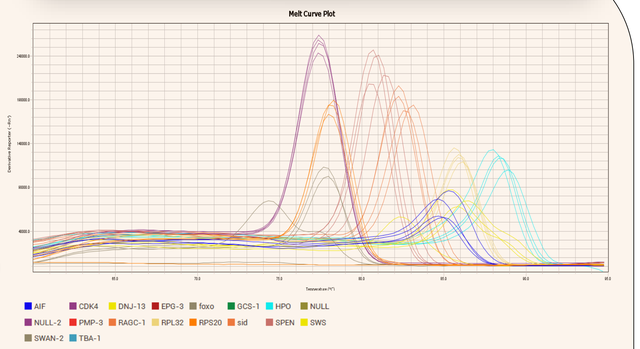

Figure 3: Melt curve plot. Peaks in light blue and lime green indicate melting temperature for *hpo* and *sws* respectively.

Figure 4: Heatmap of gene expression levels. This figure is generated with JMP Pro. Red indicates higher expression and green indicates lower expression.

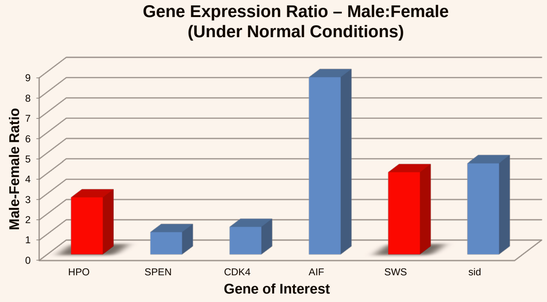

Figure 5: Male flies to female flies gene expression level fold differences. *sws* and *hpo* are highlighted in red. *sws* expression level in male flies is higher than that in female flies with about 4.06 fold difference. *hpo* was also upregulated in male flies with about 2.91 fold difference. A two fold difference in gene expression was considered statistically significant in this study.

Discussion & Conclusions

- In this study we measured the gene expression levels of *hpo* and *sws* using qRT-PCR. Our study revealed that *hpo* was expressed about 3 times more with a 0.0051 p-value and the *sws* gene was expressed about 4 times with a 0.2985 p-value more in male flies than in female flies under normal conditions.
- We conclude that there is a statistically significant upregulation of *hpo* male fruit flies compared to female fruit flies. While *sws* has a p-value > 0.05, considering the small sample size, a 4-fold difference in male and female *sws* gene expression meets the threshold of significance in this study.

Study Limitations

- This study did not account for differences between individual flies. Pooled samples from 30 fly heads were used for analysis.
- The sample size we used is relatively low and lacks statistical power.

Future Direction

- The next step in our study is to determine if the 3x increase in *hpo* gene expression in males translates to a 3x increase in the production of the *hpo* protein kinase, higher apoptosis of neurons, cardiac cells and/or insulin producing cells.
- Does stress lead to hyper-regulation or under-regulation of the *sws* gene? Possible stressors could be temperature, starvation, etc. Further study is needed to determine how gene expression differs between normal condition vs. under stress.
- Recent studies have shown that under-regulation of the Hippo signaling pathway leads to tumorigenesis and loss of homeostasis[3]. Further studies are needed to determine the lower bound on *hpo* gene expression to maintain homeostasis and prevent tumorigenesis.

Bibliography

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