Gene expression of Cdk4, a regulator for cell division, is expressed ~ 1.4-fold higher in *D. melanogaster* male heads than female heads under controlled laboratory conditions

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Summary

- *Null Hypothesis*: There is a statistically significant difference in Cdk4 gene expression levels between male and female *Drosophila melanogaster* (fruit flies) heads under unstressed conditions.
- The gene expression for the Cdk4 gene was assessed using qRT-PCR.
- Cdk4 expression was not statistically significantly different between male and female fruit flies.

Abstract

Cdk4 helps regulate cell growth and is essential for fruit fly development. This study investigated the difference in gene expression of Cdk4 between male and female fruit flies to better understand expression differences between males and females. Under controlled laboratory conditions, qRT-PCR analysis indicated that there is a 1.358-fold increase in expression of Cdk4 in males when compared to females. The quantified relative expression is below the 2-fold significance threshold. Therefore this result did not demonstrate significant gene expression differences in Cdk4 between male and female fruit flies.

Introduction

- Preliminary data shows that roughly 4% of *D. melanogaster* genes have significantly different expression patterns between genders when fed different protein to sugar ratio diets[4].
- Cdk4 gene was chosen to determine if stress could induce different gene expression levels between genders in the cell cycle pathway.
- Cdk4 codes for cyclin dependent kinase 4, a subunit of a protein complex essential for stimulating or inhibiting mitosis[5]. The Cdk4 protein regulates the G1 to S phase transition of the cell cycle when activated, or when cyclin binds to it[2].
- Controlled laboratory conditions were used to establish baseline expression.

Significance

Understanding the Cdk4 expression levels in male and female tissues and anatomical regions under control conditions will set up the baseline information for future stress experiments, and eventually give direction to research relevant to cell growth in human medicine.

Why fruit flies?

- Short lifespans and reproduce quickly in large quantities
- Clear morphological differences between sexes
- Inexpensive to raise and easily acquired
- Extensively researched and catalogued genome
- Human disease-causing genes conserved among fruit fly genome

Methodology

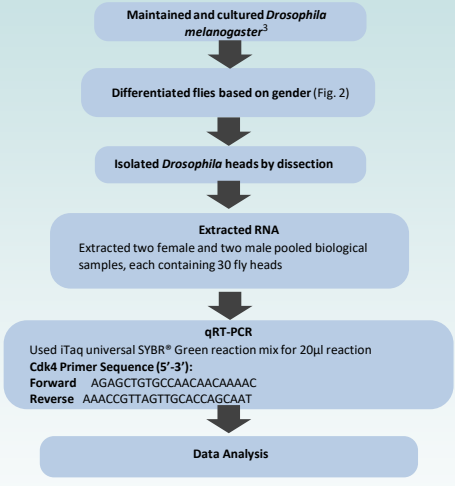

Figure 1: Flowchart of experimental design and progression.

Materials and Methods

- QIAGEN RNeasy Plus Mini Kit protocol used for RNA extraction.
- qRT-PCR primer design was determined with NCBI Primer-BLAST program[1].
- The gene RpL32, a housekeeping gene[6], was used to normalize qRT-PCR results. Normalized Ct number, ΔCt, was used to generate the heatmap.
- ΔΔCt analysis was used to generate the fold difference.
- Quality of the qRT-PCR run was checked by melt curve results.
- Gene expression data was considered to be statistically significant with a p-value of < 0.05 using an unpaired t-test and a greater than 2-fold difference.

Figure 2. Male (left) and Female (right) *Drosophila melanogaster* imaged with 30x light microscope. Sexual dimorphisms such as male specific sex combs, tergite coloration, genitalia shape, and abdomen size are highlighted in red.

Results

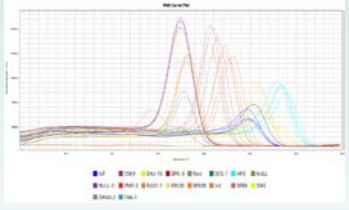

Figure 3: Melt curve plot for qRT-PCR reactions. Purple peaks, as indicated in the legend, indicate the melting temperature for the Cdk4 qRT-PCR product.

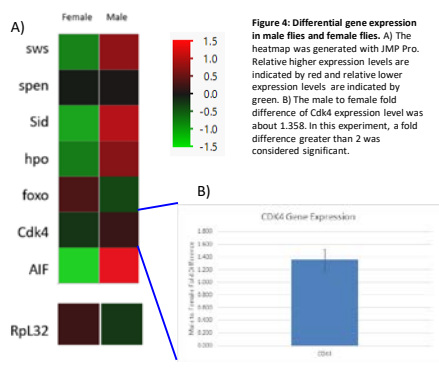

Figure 4: Differential gene expression in male flies and female flies. A) The heatmap was generated with JMP Pro. Relative higher expression levels are indicated by red and relative lower expression levels are indicated by green. B) The male to female fold difference of Cdk4 expression level was about 1.358. In this experiment, a fold difference greater than 2 was considered significant.

Discussion/Conclusion

Conclusion/Discussion:

Using standard controlled laboratory conditions have allowed us to have a baseline for the Cdk4 gene expression in fruit flies' heads. This result is consistent with Cdk4's role in regulating development and cell proliferation / differentiation. With a p-value of 0.1068, Cdk4 does not have statistically significant expression differences between males and females under unstressed conditions.

Study Limitations:

- Individual differences between flies were not considered; Two replicates of 30 flies per gender utilized in this preliminary analysis.
- Due to specimen and anatomical restrictions, small pooled sample size (N=2) did not yield a comprehensive statistical analysis.

Future Directions:

In this preliminary experiment we quantified Cdk4 gene expression levels under normal conditions to establish a baseline. Our goal is to study *D. melanogaster* gender-specific / tissue specific gene expression and genotype-phenotype correlations under various stress conditions targeting metabolic and developmental regulatory pathways. Stress conditions could include nutrient deficit, exposures to various temperatures, and isolation stressors. We strive to quantify underlying biological variation and help better understand the role of gender- and individual-specific responses to stress relative to brain physiology.

Bibliography

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