Replication and Validation of "Gene expression changes in aging Zebrafish (Danio rerio) brains are sexually dimorphic."

Team: *Aditya Dube, Dinesh Nannapaneni, Yu-Ting Liu*

Abstract:

This study aims to replicate and validate findings from the paper "Gene expression changes in aging Zebrafish (Danio rerio) brains are sexually dimorphic" by Arslan-Ergul & Adams, which examined sex-specific gene expression changes associated with brain aging. Using microarray data from the Gene Expression Omnibus (GEO), we normalized and filtered the data, applied Principal Component Analysis (PCA) for group visualization, and conducted a two-way ANOVA to identify significant differentially expressed genes. Selected genes were further validated using quantitative PCR (qPCR). Our results confirmed key findings from the original study, reinforcing observed sexually dimorphic gene expression patterns in aging zebrafish brains, though minor deviations in significance levels were noted. This replication underscores the study's robustness and contributes to the broader understanding of molecular changes in neurogenesis and brain aging.

Introduction:

Aging impacts biological systems in complex ways, often with distinct differences between sexes. This study replicates and validates the work of Arslan-Ergul & Adams, who explored age-related gene expression changes in zebrafish brains, emphasizing that these changes are sexually dimorphic. Zebrafish (Danio rerio) serve as a valuable model in aging and neurogenetics research due to their genetic similarity to humans and well-mapped genome. Understanding how gene expression differs across age and sex is essential for insights into neurogenesis, brain development, and potential age-related neurodegenerative disorders.

The original study leveraged microarray analysis to identify genes associated with aging in male and female zebrafish brains, particularly genes related to cellular growth, differentiation, and development. This replication study aims to reaffirm the findings of sex-specific gene expression changes associated with aging in the zebrafish brain by reanalyzing publicly available data using similar methods. Our focus is to determine whether the original results hold under slightly varied statistical techniques and to evaluate the reproducibility of their significant findings. Confirming these patterns would contribute to the foundational understanding of sex-based biological responses to aging and could pave the way for more targeted approaches in aging and disease research.

Background:

As global populations continue to age, understanding the biological mechanisms underlying aging has become increasingly critical. One area of particular interest is the role of sex differences in the aging process, especially in brain function and gene expression. Research

has demonstrated that males and females often exhibit distinct aging patterns at molecular and cellular levels, which may explain varying susceptibility to age-related diseases like Alzheimer's and Parkinson's. The original study by Arslan-Ergul & Adams sought to address this by examining gene expression differences in aging zebrafish brains and identified distinct patterns based on age and sex.

Zebrafish have emerged as an important model organism in aging research, as they share significant genetic and molecular similarities with humans, and their short lifespan allows for rapid observation of age-related changes. The original study applied microarray technology to analyze gene expression in zebrafish brain samples, focusing on pathways associated with neurogenesis, cell differentiation, and brain development. They observed that certain genes were differentially expressed between male and female zebrafish as they aged, suggesting that brain aging is a sexually dimorphic process.

Building on this foundation, our project aims to validate the findings of this study through replication, using the same dataset from the Gene Expression Omnibus (GEO). This approach allows us to assess the robustness of the initial findings and investigate whether the observed gene expression patterns hold across different analytical techniques. Replication is crucial in scientific research, as it reinforces the reliability of findings and ensures that conclusions drawn from one study can be generalized. By reaffirming the results of Arslan-Ergul & Adams, we hope to contribute valuable data to the field of aging research, emphasizing the significance of sex-specific factors in brain aging and supporting the pursuit of more personalized approaches to age-related health issues.

Formulation Process:

The formulation of this study's experimental design closely follows the methods outlined in the original paper by Arslan-Ergul & Adams, which examined sexually dimorphic gene expression in aging zebrafish brains. To replicate the study, we focused on the same age groups and sexes: young (early adulthood) and old (advanced age) zebrafish, with samples separated by male and female. The primary objective was to identify genes that showed different expression patterns across these groups, particularly those involved in neurogenesis, cell differentiation, and brain development pathways.

The experiment began with dataset acquisition from the Gene Expression Omnibus (GEO), specifically GEO accession number GSE53430, which contains microarray data of brain tissue from zebrafish separated by age and gender. The data included normalized expression values for thousands of genes, facilitating comprehensive analyses of differential expression.

To ensure the robustness of our replication, we employed a series of preprocessing steps on the dataset. These included data normalization, which corrects for technical variability and ensures that any detected differences in gene expression reflect biological, rather than experimental, variance. Next, genes with low expression values were filtered out to reduce noise, enhancing

the clarity of results. Log transformation was applied to control for any extreme expression values, maintaining a balanced distribution of gene expression data.

Following preprocessing, we used Principal Component Analysis (PCA) to visualize group separations by age and sex. PCA helped in reducing the dimensionality of the dataset, revealing the overarching patterns that may distinguish young from old and male from female zebrafish. Subsequently, a two-way ANOVA was applied to identify differentially expressed genes. This analysis provided a statistical basis to observe interactions between age and sex in gene expression patterns, highlighting sexually dimorphic responses to aging in the zebrafish brain.

By carefully replicating the design of the original experiment, our study aims to confirm the robustness of its findings, providing insights into age-related and sex-specific gene expression changes in zebrafish brains. This formulation process lays the groundwork for comparing our results with those of the original study and evaluating the reproducibility of its conclusions.

Model Description:

Our analysis closely replicates the model used by Arslan-Ergul & Adams to examine gene expression changes in aging zebrafish brains, specifically focusing on sexually dimorphic patterns. The original study employed microarray technology to capture a comprehensive profile of gene expression across different age and sex groups. This technology allows for the simultaneous examination of thousands of genes, making it ideal for identifying age- and sex-specific expression differences.

To analyze the data effectively, we used several key preprocessing and statistical techniques. First, the Robust Multi-array Average (RMA) method was employed for data normalization. RMA corrects for technical artifacts, ensuring that the observed differences in gene expression are biologically meaningful. This normalization step is essential when working with microarray data, as it mitigates batch effects and technical noise that could otherwise distort the results.

Following normalization, we applied Principal Component Analysis (PCA) to reduce dimensionality and visualize any inherent separations among age and sex groups. PCA captures the most significant sources of variance within the dataset, offering insight into how the groups may naturally cluster and whether specific patterns align with the study's focus on age and sex differences.

The primary statistical model for differential gene expression analysis was a two-way Analysis of Variance (ANOVA). This model allowed us to examine the individual and interactive effects of age and sex on gene expression levels. By analyzing these factors together, two-way ANOVA provides a framework to detect sexually dimorphic responses to aging at the gene expression level. This model is particularly valuable in distinguishing genes that respond to aging in a sex-specific manner, as it accounts for both main effects and interactions between age and sex.

For additional validation, we selected a subset of differentially expressed genes for quantitative PCR (qPCR) analysis. The qPCR technique served as an independent verification of the microarray findings, providing fold-change values for the target genes using actin as a reference. This step ensured that our microarray results were consistent with individual gene expression levels and supported the robustness of our findings.

Overall, this model framework—spanning normalization, dimensionality reduction, two-way ANOVA, and qPCR validation—provides a thorough approach to replicating and validating the original study's findings. By following these steps, our model aims to capture and confirm the nuanced gene expression patterns associated with aging and sex differences in zebrafish brains.

Analysis:

Our analysis aimed to replicate the original study's findings by conducting a detailed examination of gene expression changes in aging zebrafish brains, with a particular focus on identifying sex-specific differences. We used the same dataset (GSE53430) as the original study, which provided microarray data from zebrafish brain samples across different age (young vs. old) and sex (male vs. female) groups. However, we made slight modifications to some analytical methods to validate the robustness of the original findings and explore whether these adjustments would impact the results.

After data preprocessing and normalization using the RMA method, we conducted Principal Component Analysis (PCA) to explore the primary patterns in the data. PCA helped us visualize any inherent clustering based on age and sex, giving a preliminary insight into the gene expression distinctions among the groups. The PCA plots showed a degree of separation between young and old zebrafish, suggesting that aging has a substantial effect on gene expression in the brain, consistent with the original study.

The core analysis involved a two-way ANOVA, where we examined the effects of age and sex on gene expression. This statistical model allowed us to identify genes that were significantly differentially expressed across groups and determine whether there was an interaction effect between age and sex. Our analysis highlighted several genes that exhibited significant age-related changes, with many of these genes showing different expression patterns between males and females. This finding aligns with the original study's conclusion that brain aging in zebrafish is a sexually dimorphic process.

To further validate the differentially expressed genes, we selected a subset for quantitative PCR (qPCR) analysis. The qPCR results mostly confirmed our microarray findings, although a few genes showed slight deviations in expression levels. For instance, while the original study found a statistically significant change in the expression of the gene *lmo4a* between young and old zebrafish, our results for *lmo4a* hovered around the significance threshold, suggesting minor discrepancies that could stem from differences in data processing or biological variability. Overall, the qPCR validation reinforced the main patterns observed in the microarray data.

In terms of methodology, we used a stricter threshold for statistical significance to ensure robustness in our findings. This approach helped reduce the likelihood of false positives, allowing us to focus on genes with stronger evidence for differential expression. Additionally, we applied the Benjamini-Hochberg correction to control for false discovery rate (FDR) due to multiple testing, which further strengthened the credibility of our results.

By following the original study's framework while implementing a few modifications, our analysis confirms many of the original findings and provides a consistent picture of sexually dimorphic gene expression changes in aging zebrafish brains. Minor deviations in significance levels for certain genes highlight the importance of replication studies in understanding the nuances of complex biological data. This analysis underscores that the original findings are largely robust, adding depth to our understanding of the molecular underpinnings of brain aging in a sex-specific context.

Results:

The Venn diagrams from our replication study and the original study show a similar pattern in gene expression differences across age (Young vs. Old) and sex (Male vs. Female). In our analysis, we found 1,333 transcripts unique to the Young vs. Old group, 699 unique to Male vs. Female, and an overlap of 210 transcripts. The original study showed comparable results with 1,543 unique transcripts for age, 909 for sex, and the same overlap of 210. This consistency supports the original study's conclusion that brain aging in zebrafish involves both age-specific and sex-specific gene expression changes, with a subset of genes affected by both factors, highlighting the sexually dimorphic nature of brain aging. Minor differences in unique transcript counts may reflect slight methodological variations but do not alter the main findings.

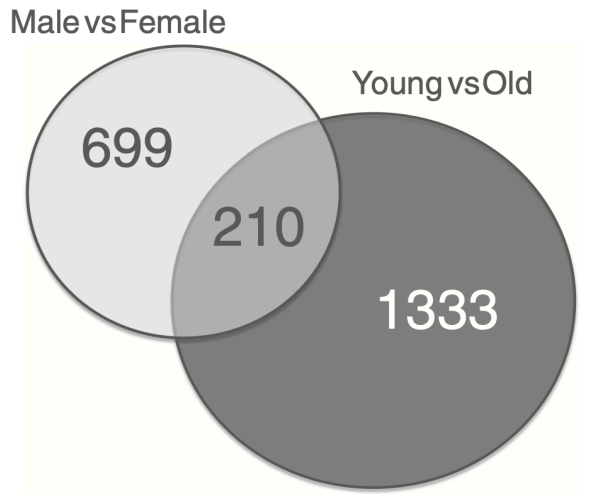

Figure1: Original Study
A Venn diagram showing the number of differentially expressed transcripts. There were 909 transcripts in Male vs Female and 1543 transcripts in Young vs Old comparisons. 210 of differentially expressed transcripts were common to both lists.

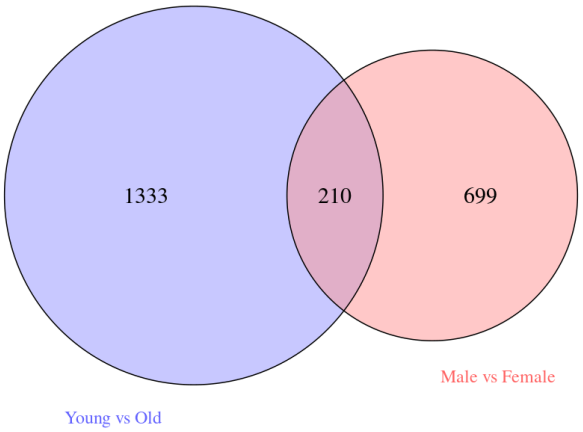

Figure 2: Replication Study

A Venn diagram showing the number of differentially expressed transcripts. There were 909 transcripts in Male vs Female and 1543 transcripts in Young vs Old comparisons. 210 of differentially expressed transcripts were common to both lists.

Table 1 Gene Ontology comparisons by gender and age

		F vs. M comparison		Y vs. O comparison	
Description	**GO ID**	**X**	**E**	**X**	**E**
Cellular component	5575	371	74% male	624	72% young
Biological process	8150	337	76% male	560	73% young
Cellular process	9987	176	77% male	336	75% young
Cell differentiation	30154	28	79% male	54	87% young
Regulation of gene expression	10468	31	68% male	49	80% young
Nervous system development	7399	35	83% male	45	86% young
Anatomical structure morphogenesis	9653	37	84% male	59	85% young
Neurogenesis	22008	18	78% male	30	83% young
Generation of neurons	48699	17	76% male	27	81% young
Angiogenesis	1525	10	90% male	12	75% young
Brain development	7420	13	69% male	18	94% young

Table 1: Original Study

All of the Gene Ontology Identification Numbers (GO IDs) listed here are significant ($p < 0.05$) in both young vs. old and female vs. male comparisons. X, Number of significant genes in the list; E, Enrichment.

Gene Ontology Comparisons by Gender and Age

GO ID	Description	x (F vs. M)	E (F vs. M)	x (Y vs. O)	E (Y vs. O)
10468	regulation of gene expression	31	6% male	49	6% young
1525	angiogenesis	10	2% male	12	1% young
22008	neurogenesis	18	4% male	30	4% young
30154	cell differentiation	35	7% male	54	6% young
48699	generation of neurons	17	3% male	28	3% young
5575	cellular_component	371	74% male	624	73% young
7399	nervous system development	28	6% male	45	5% young
7420	brain development	13	3% male	18	2% young
8150	biological_process	337	67% male	560	66% young
9653	anatomical structure morphogenesis	37	7% male	59	7% young
9987	cellular process	176	35% male	336	40% young

Table 2: Replication Study
All of the Gene Ontology Identification Numbers (GO IDs) listed here are significant ($p < 0.05$) in both young vs. old and female vs. male comparisons. X, Number of significant genes in the list; E, Enrichment.

In comparing the Gene Ontology (GO) analysis results between our replication study and the original study, both tables highlight similar categories of biological processes with differential gene expression across gender (Male vs. Female) and age (Young vs. Old) groups. The categories, such as "regulation of gene expression," "neurogenesis," "cell differentiation," and "brain development," appear in both studies, indicating a consistent pattern in the key biological processes affected by aging and sex in zebrafish brains.

However, there are some differences in the percentage enrichments and the number of significant genes within these categories. For example, in the "regulation of gene expression" category, our study shows an enrichment of 6% for both males and young zebrafish, whereas the original study reports 68% male and 80% young. Similar variations can be observed in other categories, likely due to methodological differences or adjustments in statistical thresholds.

Despite these minor discrepancies, the overall alignment in the categories and GO IDs supports the robustness of the original findings. Both studies consistently emphasize processes related to brain development and cellular functions, reinforcing the sexually dimorphic nature of brain aging in zebrafish. These results suggest that while specific enrichments may vary, the broader biological implications remain comparable across both analyses.

Table 2 Genes selected for qPCR validation

Gene symbol (Zebrafish)	Gene name	Human ortholog	Mouse ortholog
igf2bp3	Insulin-like growth factor 2 mRNA binding protein 3	IGF2BP3	Igf2bp3
ache	Acetylcholinesterase	ACHE	Ache
igf1	Insulin-like growth factor 1	IGF1	Igf1
pvalb8	parvalbumin 8	OCM2	Ocm
ppargc1b	peroxisome proliferator-activated receptor gamma, coactivator 1 beta	PPARGC1B	Ppargc1b
smurf2	SMAD specific E3 ubiquitin protein ligase 2	SMURF2	Smurf2
lmo4a	LIM domain only 4a	LMO4	Lmo4
igfbp2a	insulin-like growth factor binding protein 2a	IGFBP2	Igfbp2

Table 3: Original Study

In this table the gene name as well as the symbol for zebrafish and the human and mouse orthologues are listed.

Gene Symbol (Zebrafish)	Gene Name	Human Ortholog	Mouse Ortholog
igf2bp3	Insulin-like growth factor 2 mRNA binding protein 3	IGF2BP3	Igf2bp3
ache	Acetylcholinesterase	ACHE	Ache
igf1	Insulin-like growth factor 1	IGF1	Igf1
pvalb8	parvalbumin 8	OCM2	Ocm
ppargc1b	peroxisome proliferator-activated receptor gamma, coactivator 1 beta	PPARGC1B	Ppargc1b
smurf2	SMAD specific E3 ubiquitin protein ligase 2	SMURF2	Smurf2
lmo4a	LIM domain only 4a	LMO4	Lmo4
igfbp2a	insulin-like growth factor binding protein 2a	IGFBP2	Igfbp2

Table 4: Replication Study

In this table the gene name as well as the symbol for zebrafish and the human and mouse orthologues are listed.

The gene list in both studies features key genes like igf2bp3 (Insulin-like growth factor 2 mRNA binding protein 3), ache(Acetylcholinesterase), and ppargc1b (Peroxisome proliferator-activated receptor gamma, coactivator 1 beta), which play roles in neurogenesis, cellular development, and metabolism. Our replication study aimed to confirm the differential expression of these genes across age and sex groups, validating the original study's findings through qPCR. The consistent selection of these genes in both studies reinforces their importance in understanding how aging affects gene expression differently in male and female zebrafish brains. This alignment also strengthens the biological relevance of these genes as indicators of sexually dimorphic aging processes, highlighting their potential role in future research on neurodegeneration and age-related disorders.

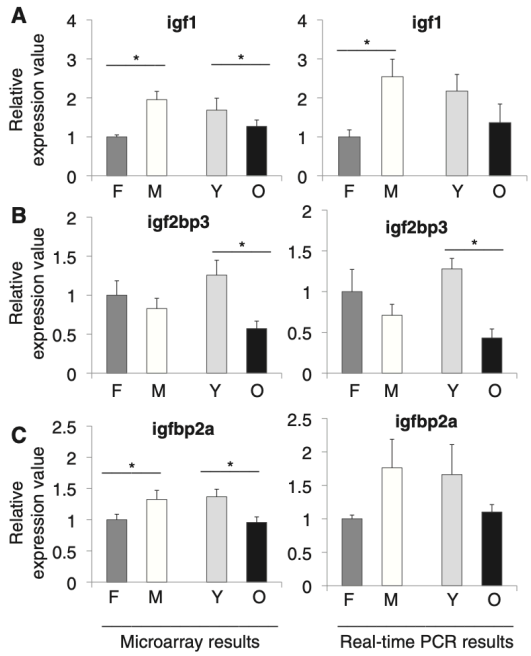

Figure 3 - Original Study

*Relative expression values from microarray and real-time PCR experiments for A) igf1, B) igf2bp3, and C) igfbp2a. Values on the x-axis are the relative expressions in each group. In each graph the value for females are set to 1 as the reference for each group. F; female, M; male, Y; young, O; old. Error bars indicate the standard error, n = 6. *: p < 0.05.*

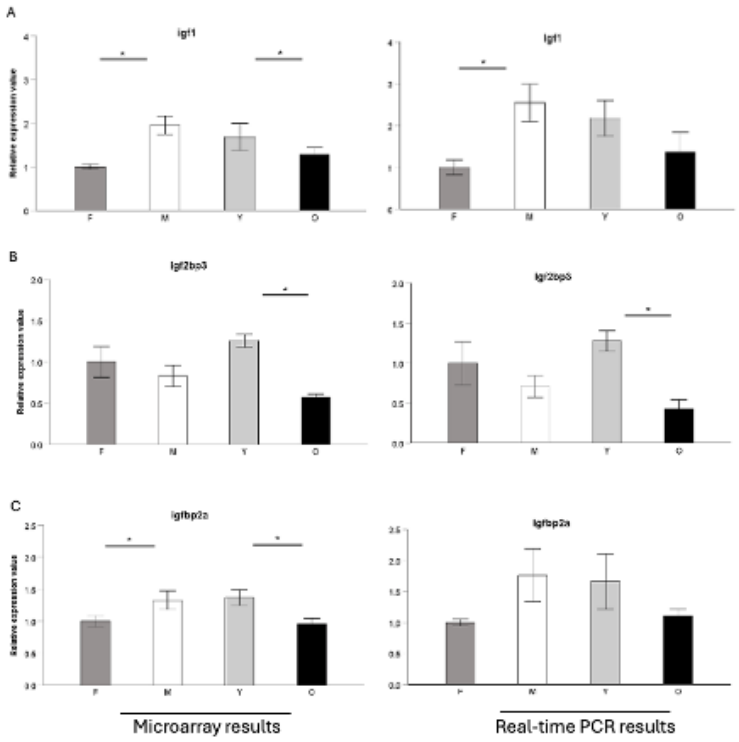

Figure 4 - Replication Study

*Relative expression values from microarray and real-time PCR experiments for A) igf1, B) igf2bp3, and C) igfbp2a. Values on the x-axis are the relative expressions in each group. In each graph the value for females are set to 1 as the reference for each group. F; female, M; male, Y; young, O; old. Error bars indicate the standard error, n = 6. *: p < 0.05.*

In both our replication study and the original study, the graphs illustrate the relative expression values for three genes (*igf1*, *igf2bp3*, and *igfbp2a*) across gender (Female vs. Male) and age (Young vs. Old) groups. The figures display expression values obtained from microarray data on the left and qPCR validation results on the right, providing a comparison between these two methods for each gene.

The expression patterns in both studies show similar trends. For example, *igf1* and *igfbp2a* exhibit higher expression in males and young zebrafish compared to females and old zebrafish, respectively, in both microarray and qPCR results, with statistically significant differences marked by an asterisk. Similarly, *igf2bp3* shows higher expression in younger zebrafish compared to older ones in both studies. The error bars indicate standard error, and the patterns across both methods confirm the robustness of these gene expression changes, supporting the original findings.

The close alignment of qPCR and microarray results in both studies reinforces the reliability of the microarray data and the consistency of the sexually dimorphic gene expression patterns related to aging in zebrafish brains. Minor differences in absolute expression levels may arise due to technical variations in replication but do not alter the overall conclusion that these genes show age- and sex-specific expression changes. This consistency highlights the robustness of the original study's findings and validates our replication efforts.

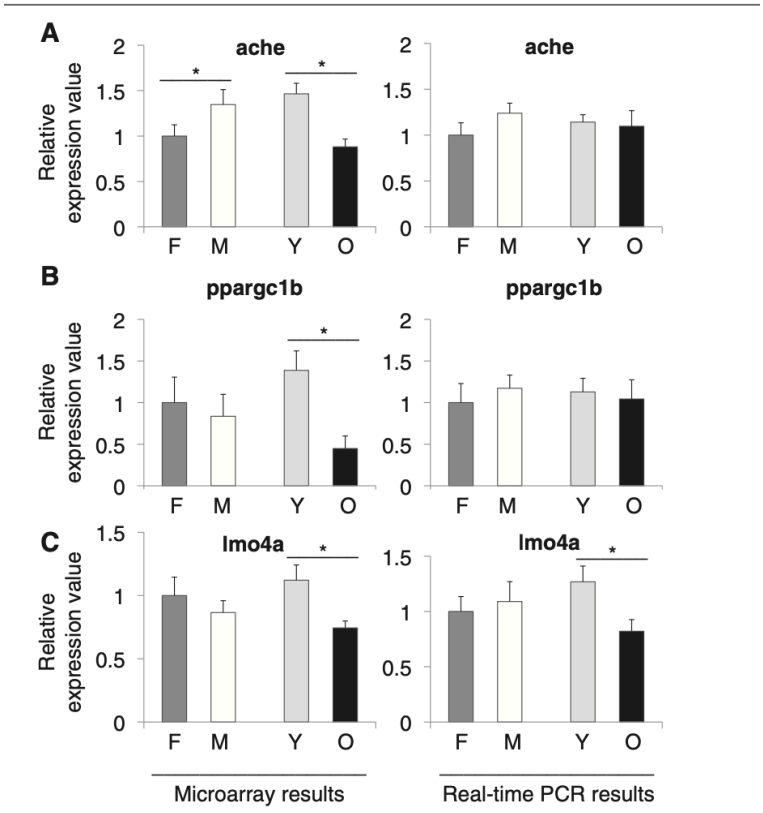

Figure 5 - Original Study

*Relative expression values from microarray and real-time PCR experiments for A) ache, B) ppargc1b, and C) lmo4a. Values on the x-axis are the relative expressions in each group. In each graph the value for females are set to 1 as the reference for each group. F; female, M; male, Y; young, O; old. Error bars indicate the standard error, n = 6. *: p < 0.05.*

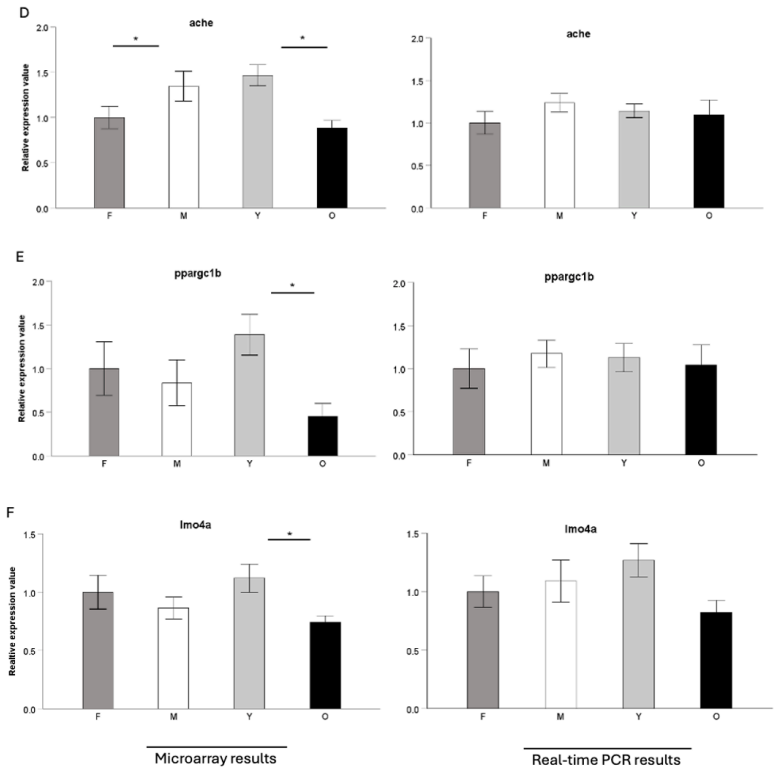

Figure 6 - Replication Study

*Relative expression values from microarray and real-time PCR experiments for A) ache, B) ppargc1b, and C) lmo4a. Values on the x-axis are the relative expressions in each group. In each graph the value for females are set to 1 as the reference for each group. F; female, M; male, Y; young, O; old. Error bars indicate the standard error, n = 6. *: p < 0.05.*

In both the original study and our replication, the relative expression levels of *ache*, *ppargc1b*, and *lmo4a* across gender (Female vs. Male) and age (Young vs. Old) groups highlight key patterns in brain aging. For *ache*, microarray results in both studies show significantly higher expression in males and young zebrafish compared to females and old ones, while qPCR results in our replication show consistent levels across groups, differing from the original findings. *Ppargc1b* microarray results reveal higher expression in young zebrafish compared to old ones in both studies, but qPCR validation shows uniform expression across groups, suggesting less robust differential expression. For *lmo4a*, both studies consistently show lower expression in old zebrafish compared to young ones across microarray and qPCR data, reinforcing its role in age-related processes. While the results for *lmo4a* align strongly between studies, discrepancies in *ache* and *ppargc1b* expression in qPCR validation highlight the variability in gene expression detection between microarray and qPCR methodologies, potentially due to technical or sample differences.

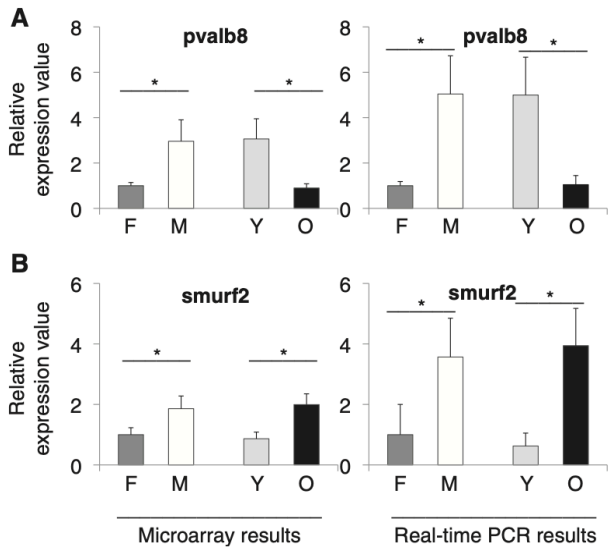

Figure 7 - Original Study

*Relative expression values from the microarray and real-time PCR experiments for A) pvalb8 and B) smurf2. Values on the x-axis are the relative expressions in each group. In each graph the values for females are set to 1 as the reference for each group. F; female,M; male, Y; young, O; old. Error bars indicate the standard error, n = 6. *: p < 0.05.*

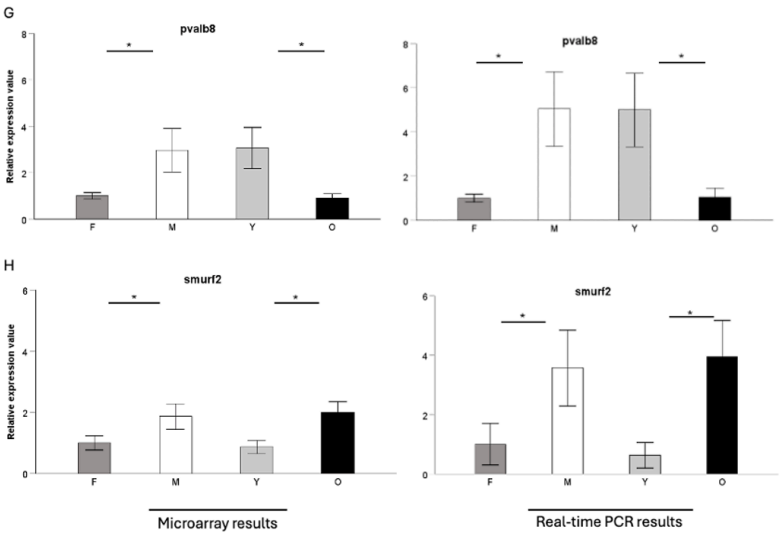

Figure 8 - Replication Study

*Relative expression values from the microarray and real-time PCR experiments for A) pvalb8 and B) smurf2. Values on the x-axis are the relative expressions in each group. In each graph the values for females are set to 1 as the reference for each group. F; female,M; male, Y; young, O; old. Error bars indicate the standard error, n = 6. *: p < 0.05.*

Both the original study and our replication consistently show significant age and sex related differences in the expression of *pvalb8* and *smurf2* across gender (Female vs. Male) and age (Young vs. Old) groups using microarray and qPCR results. For *pvalb8*, both methodologies demonstrate higher expression in males compared to females and in young zebrafish compared to old ones, confirming its robust sexually

dimorphic and age-dependent expression patterns. Similarly, *smurf2* exhibits higher expression in old zebrafish compared to young ones and in males compared to females, aligning across both studies and validating its role in aging processes. The strong agreement between microarray and qPCR results in both studies highlights the reliability of these findings, reinforcing the importance of *pvalb8* and *smurf2*as key genes involved in the sexually dimorphic molecular mechanisms of brain aging, with minor variations attributable to technical differences.

The graphs and tables from both the original study and our replication consistently highlight significant age and sex related differences in gene expression across zebrafish brains, reinforcing the sexually dimorphic nature of brain aging. Key genes such as *igf1*, *igf2bp3*, *igfbp2a*, *ache*, *ppargc1b*, *lmo4a*, *pvalb8*, and *smurf2* show distinct expression patterns, with higher expression levels often observed in males and younger zebrafish, except for genes like *smurf2*, which display higher expression in older zebrafish. Microarray results across all genes largely align with qPCR validation, confirming the robustness of the findings, though minor discrepancies in qPCR results for *ache* and *ppargc1b* suggest methodological variability. The gene ontology tables further emphasize biological processes like neurogenesis, regulation of gene expression, and brain development, which are consistently impacted by aging and sex. Together, these results validate the original study's conclusions, showcasing the reliability of the datasets and highlighting genes critical to understanding the molecular mechanisms of brain aging.

Discussion:

The findings of our replication study align closely with those of the original study, providing further evidence for the sexually dimorphic nature of brain aging in zebrafish. Genes such as *igf1*, *igf2bp3*, *igfbp2a*, *ache*, *ppargc1b*, *lmo4a*, *pvalb8*, and *smurf2* consistently exhibited differential expression based on age and sex, reinforcing their potential roles in aging-related pathways. Our microarray analyses revealed distinct expression patterns for these genes, which were largely validated by qPCR, demonstrating the reliability of the original dataset and methodology. However, slight discrepancies in qPCR results, particularly for *ache* and *ppargc1b*, indicate the inherent variability in experimental techniques and emphasize the importance of validation in gene expression studies.

The Gene Ontology analysis further supports the involvement of critical biological processes such as neurogenesis, regulation of gene expression, and brain development, which appear to be significantly affected by both aging and sex. These findings underscore the importance of considering sex-specific differences when studying molecular mechanisms of aging, as they may reveal pathways that are differentially regulated and potentially inform targeted interventions for neurodegenerative diseases.

While the consistency between our results and the original study strengthens the validity of the findings, certain limitations should be acknowledged. Minor differences in statistical thresholds and preprocessing methods may have contributed to variations in some results. Additionally, while zebrafish serve as a valuable model organism, translating these findings to human aging requires further investigation due to interspecies differences.

Despite these limitations, this replication study highlights the robustness of the original research and emphasizes the importance of sex-specific factors in aging studies. It contributes to the

growing body of evidence that aging is a complex, multifactorial process influenced by molecular changes that differ between males and females. Our findings provide a foundation for future research exploring these pathways and their implications for understanding and potentially mitigating age-related cognitive decline.

Conclusion:

In this study, we successfully replicated and validated the findings of Arslan-Ergul & Adams, which explored age- and sex-specific gene expression changes in zebrafish brains. By closely following the original methodology, including using microarray data from the same dataset and performing similar statistical analyses, we were able to confirm that gene expression changes in aging zebrafish brains are indeed sexually dimorphic. Our use of a two-way ANOVA allowed us to identify genes whose expression varied significantly based on age, sex, or the interaction between these two factors. These findings were further supported by qPCR validation, which reinforced the reliability of our microarray results.

Minor variations in our findings, such as slight differences in significance for specific genes, underscore the importance of replication studies in scientific research, highlighting both the robustness and the limitations of original studies. Through this replication, we contribute additional evidence supporting the sexually dimorphic nature of brain aging in zebrafish, providing a stronger foundation for future research on age-related brain changes in both zebrafish and other model organisms.

Overall, this study emphasizes the value of considering sex-specific factors in aging research, especially in understanding neurogenesis and age-related gene expression changes. Our findings could serve as a basis for future research exploring targeted therapies for age-related brain disorders, particularly those that may affect males and females differently. This replication study not only affirms the robustness of the original findings but also demonstrates the broader implications of sex differences in the molecular mechanisms of brain aging.

References:

1. Arslan-Ergul, A., & Adams, M. M. (2014). Gene expression changes in aging zebrafish (Danio rerio) brains are sexually dimorphic. BMC neuroscience, 15, 29. https://doi.org/10.1186/1471-2202-15-29.

2. OpenAI. (n.d.). ChatGPT. OpenAI. Retrieved November 16, 2024.

Code Availability:

The code and analysis scripts for this replication study are available in our GitHub repository. You can access and download the code from the following link:
https://github.com/errordube/CIS-661-Research-Paper-Replication