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■? ARTICLE

Natural Genetic Variation in Mitochondrial Health-Regulating Genes in the *C. Elegans* Strains CX11314 and EG4725 Leads to Increased Mitochondrial Resilience.

Aaryan Samanta[1], Mansi Mandal[2], Mohammed Yasar Meeran[3]

1) Legend College Preparatory School; aaryan.samanta.0@gmail.com
2) Mountain House High School,; mansimandal999@gmail.com
3) Lebanon Train High School; yasar.meeran@gmail.com

ABSTRACT: Mitochondria play a central role in maintaining cellular function and homeostasis, and their dysfunction can severely impact lifespan and aging. Owing to their conserved pathways, human orthologs, and experimental tractability, *Caenorhabditis elegans* was used as the model organism in this study. We investigated natural genetic variation in the *C. elegans* strains CX11314 and EG4725, which are characterized by increased resistance to mitochondrial dysfunction. Specifically, we examined polymorphisms and differential expression in six key mitochondrial health–regulating genes—*atfs-1, eat-3, fzo-1, pdr-1, drp-1,* and *pink-1*—that control mitochondrial morphology and mitophagy, using variant analysis, phylogenetic comparison, and RNA-seq data. Our analyses identified strain-specific SNPs in these genes and revealed differences in their expression levels, suggesting that natural genetic variation may modulate mitochondrial resilience. Building on these findings, we propose applying CRISPR–Cas9–mediated homology-directed repair (HDR) genome editing to directly test how specific variants in mitochondrial-regulating genes give rise to distinct phenotypic and behavioral outcomes.

KEYWORDS: *C. elegans*, Mitochondrial Dysfunction, Mitophagy, Mitochondrial Morphology, Mitochondrial Resilience.

■ Introduction

According to the Cleveland Clinic, approximately 1 in every 5,000 individuals is affected by a genetic mitochondrial disease.[1,2] These diseases can affect nearly every organ, ranging from the brain and nervous system to the heart and liver, emphasizing the importance of analyzing these conditions through the various related genes and genetic pathways.

Its transparent body allows scientists to easily observe internal structures and track subtle changes in development, behavior, and gene expression with ease.[3] Its short lifespan helps researchers quickly observe the effects of genetic or environmental changes across an entire lifespan, making experiments more efficient. Additionally, since *C. elegans* are hermaphrodites, they will be able to produce offspring through self-fertilization. This minimizes genetic variation in the tests since all offspring will be genetically identical across generations. Furthermore, researchers can directly translate discoveries made with *C. elegans* to human diseases, as there are conserved genetic pathways and functional orthologs.

It is crucial to note that varying *C. elegans* strains should be used, as the majority of studies pertaining to *C. elegans* have been conducted on the N2 (Bristol) strain.[4] However, conducting research on strains with unique abilities like heteroplasmy resistance will reveal the genetic variations that result in that characteristic. Therefore, this study will focus on two *C. elegans* strains that have shown improved mitochondrial function and resilience: CX11314 and EG4725. By identifying genes and expression pathways that contribute to their improved mitochondrial morphology and mitophagy, we aim to discover novel insights into the biological mechanisms that promote healthy aging. These discoveries will then have the potential to facilitate research into age-related decline in humans.

Previous research has found that mitochondrial dysfunction accumulates as individuals age, and removing functionally compromised mitochondria helps organisms recover from the adverse effects.[5] In fact, research in fruit flies has shown that by removing damaged mitochondria, male and female fruit flies were able to live 20 percent and 12 percent longer, respectively. Additionally, previous studies have discovered cellular mechanisms that maintain mitochondrial health and slow down the aging process. In the N2 *C. elegans* strain, mitochondrial fission genes (*drp-1*), fusion genes (*fzo-1* and *eat-3*), mitophagy genes (*pink-1* and *pdr-1)*, and mitochondrial unfolded protein response (UPR) genes (*atfs-1*) have been identified. These findings have identified that these genes assist in maintaining mitochondrial structure, responding to stress, and removing damaged mitochondria.[6-8] However, when these processes fail, cells accumulate dysfunctional mitochondria, contributing to aging, neurodegeneration, and other chronic diseases.[9] As mitochondrial dysfunction continues to emerge as a key factor in many human diseases, understanding the genetic control of mitochondrial maintenance is more relevant than ever. Yet, despite these advances, most research relies on lab-adapted strains and overlooks the natural genetic diversity that could reveal new, potentially therapeutic, pathways to promote mitochondrial resilience and healthy aging. As shown in Figure 1, the protein uaDf5 shows varied expression across the wild isolate strains, indicating significant diversity in protein levels.

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Future studies are also focusing on studying the evolutionary adaptations and genetic differences between different strains of *C. elegans*. This shift from single-strain to multi-strain comparisons can help researchers uncover many evolutionary adaptations in *C. elegans* that are caused by the different environmental pressures. In a study by Zhang *et al.*, researchers analyzed gene expression across 207 wild *C. elegans* isolates and showed that natural diversity in gene expression and possible regulatory mechanisms highlight the promise of using gene expression variation to understand how phenotypic diversity is generated.[10] Furthermore, recent research projects have started to use natural genetic variation in *C. elegans* strains as a tool for discovering new aging regulators. For example, a paper by Yin *et al.* discusses how different *C. elegans* isolates show diverse lifespans and age-related declines. In the study, they identified natural genetic factors that influenced the rate of aging in *C. elegans* by looking at the different aging mechanisms among the different *C. elegans* isolates and comparing them to their genetic variance.[11]

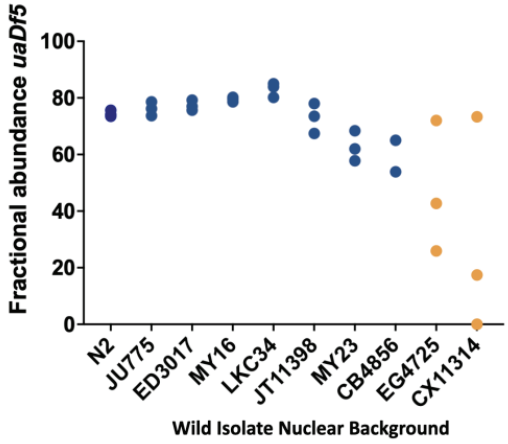

Figure 1: The fractional abundance of the protein uaDf5 for each wild isolate strain. This figure is based on unpublished data provided by the Rothman Laboratory at UCSB. This variation highlights substantial diversity in mitochondrial-related protein abundance among wild *C. elegans* strains.

The field of aging genetics in *C. elegans* has directed its focus on mito-nuclear interactions and post-transcriptional control under stress. Recent studies have also found that these interactions regulate mitochondrial function and aging. Furthermore, lifespan is a polygenic trait heavily influenced by approximately 293 nuclear regions as well as widespread mito-nuclear interactions, with worms with enhanced interactions living significantly longer.[12] In recent years, studies have been utilizing gene expression data for analysis. Unfortunately, there remains a major gap in understanding how SNPs and varying gene expression improve mitochondrial health. As illustrated in Figure 2, mitochondrial quality control involves coordinated regulation of nuclear and mitochondrial genes governing fusion, fission, mitophagy, and stress response.

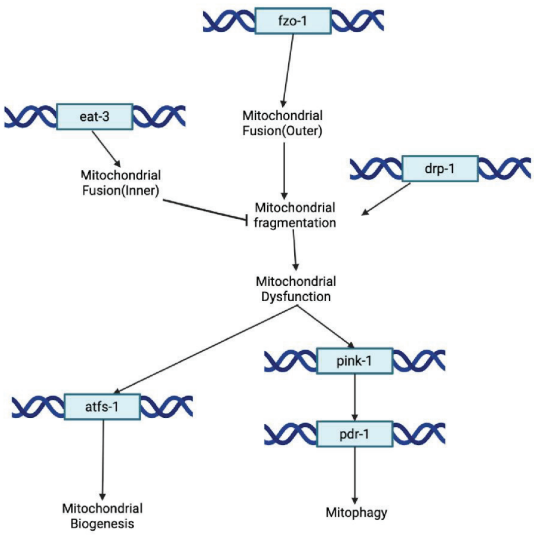

Figure 2: Overview of mitochondrial quality control pathways with mitochondrial and nuclear genes. These pathways collectively maintain mitochondrial integrity and cellular homeostasis.

This leads to our research question: What genetic variants in mitochondrial morphology and mitophagy-related genes contribute to the regulation of mitochondrial quality and heteroplasmy in the *C. elegans* strains CX11314 and EG4725 compared to the N2 reference strain? We hypothesize that genetic variation and variable expression in mitochondrial morphology and mitophagy-regulating genes (*drp-1*, *fzo-1*, *pink-1*, *eat-3*, *pdr-1*, and *atfs-1*) enhance mitochondrial quality control in the wild *C. elegans* strains CX11314 and EG4725, leading to reduced mitochondrial dysfunction and lower levels of heteroplasmy compared to the N2 reference strain. Our study aims to answer these questions by analyzing genomic variants and expression levels through the utilization of bioinformatics. As depicted in Figure 3, our methodology combines variant analysis, gene expression analysis, and functional testing to assess mitochondrial health in the strains.

Methods

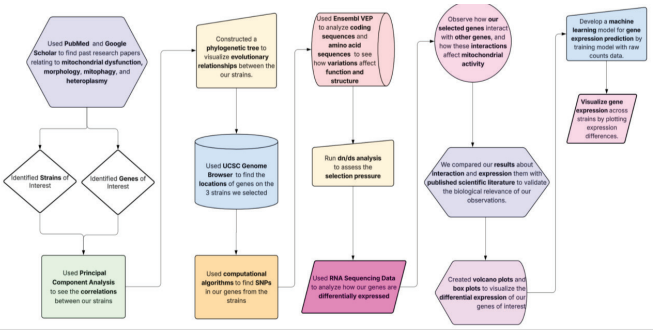

Figure 3: Methodological workflow used in this study. The workflow includes literature-based gene selection, CaeNDR variant analysis, PCA and phylogenetic comparison, VEP and dN/dS analyses, RNA-seq expression analysis, machine-learning-based EG4725 expression prediction, and proposed CRISPR-Cas9 functional validation.

To investigate the genetic differences underlying mitochondrial function in *C. elegans* strains, we designed a stepwise bioinformatics workflow that combined literature review, variant analysis, phylogenetic comparison, differen-

tial gene expression analysis, and functional interpretation. First, PubMed, PMC, Europe PMC, and Google Scholar were searched for studies related to mitochondrial morphology, mitophagy, mitochondrial dysfunction, and heteroplasmy. Based on this review, we selected the N2 reference strain and the CX11314 and EG4725 strains for further analysis because CX11314 and EG4725 have been associated with improved mitochondrial function compared with the standard N2 strain. We then focused on six genes involved in conserved mitochondrial quality-control processes: atfs-1, eat-3, fzo-1, pdr-1, drp-1, and pink-1. Comparing these strains with N2 allowed us to identify genetic and expression-level differences that may contribute to mitochondrial resilience.

After obtaining Variant Call Format (VCF) files from CaeNDR, a *C. elegans* natural diversity resource, bioinformatics Python libraries such as scikit-allel (v1.3.13), Biopython (v1.85), ETE3 (v3.1.3), PyDESeq2 (v1.40.2), NumPy (v2.3.2), Matplotlib (v3.10.8), and SciPy (v1.16.0) were utilized for further analysis.[13-20] Principal Component Analysis (PCA), a statistical method commonly used to simplify complex, high-dimensional data while preserving variance, was used to compare variants between 540 isotypes. This visualization helped assess the genetic similarities, where the distance between each strain increases with genetic divergence.

A phylogenetic tree was then used to visualize the overall evolutionary relationships and genetic distances among the 12 selected *C. elegans* isotypes: MY1, AB1, EG4725, CB4856, N2, MY16, JU393, ED3017, DL238, LKC34, GXW1, and CX11314. The UCSC genome browser, an online tool that allows scientists to analyze DNA from different organisms, was used to find the locations of our selected genes in the *C. elegans* genome. The gene ranges obtained from the genome browser helped us identify genetic differences in our strains of interest by consulting the VCF data.

To identify differentially expressed genes, we processed RNA-seq counts data for DESeq2 analysis, comparing the CX11314 and N2 strains. Using the VCF data, we identified SNPs where the strains differed and generated variant tables, helping us pinpoint mutations that could affect gene function and explain expression differences from our RNA-seq results.

The Ensembl Variant Effect Predictor (VEP) was used to compare DNA, RNA, and protein sequences and identify nucleotide and amino acid differences.[21] This analysis helped us examine how genetic variants may affect protein structure and function, thereby influencing mitochondrial health. To determine whether the observed genetic differences in the genes of interest were likely shaped by selection, the nonsynonymous/synonymous substitution ratio (dN/dS) was calculated for each candidate gene.

Gene expression data from 609 samples across 208 strains were obtained from the NCBI GEO dataset GSE186719, which includes raw read counts and TPM (Transcripts per million) values.[22] These data were used to examine expression differences in mitochondrial-related genes between CX11314 and N2 and to approximate EG4725-related expression patterns. Transcript isoforms were identified using WormBase, which provides transcript names, genomic coordinates, and functional annotations. Differential expression analysis was performed using PyDESeq2 on raw read counts, while TPM values were used for visualization. Because EG4725 was not included in GSE186719, NIC266 was selected as a proxy strain based on genetic similarity. Earlier PCA analysis of 540 isotypes identified NIC266 as the closest strain to EG4725, and VCF analysis showed very few SNP differences between NIC266 and EG4725. Therefore, RNA-seq expression data from NIC266 were used in place of EG4725 for differential expression analysis and visualization, while this limitation was clearly considered during interpretation.

Following the DESeq2 analysis, differentially expressed genes were visualized using volcano plots and box plots. Volcano plots highlighted genes with statistically significant fold changes using a Benjamini-Hochberg (BH) adjusted p-value threshold of < 0.05.[23] Box plots generated from TPM values showed the distribution of expression levels for selected genes across N2, CX11314, and NIC266. Because direct RNA-seq expression data for EG4725 were unavailable, a machine-learning approach was used to predict its gene expression profile. After data cleaning, a neural network with leave-one-out cross-validation (LOOCV) was developed; NIC266 was left out during validation because it is genetically closest to EG4725. The model was implemented using Python packages including Keras (v3.10.0), TensorFlow (v2.19.0), scikit-learn (v1.7.1), and SciPy (v1.16.0). Spearman's correlation was then used to evaluate the confidence of the EG4725 expression prediction.24 Finally, gene interaction patterns were reviewed and cross-referenced with existing literature to interpret the biological relevance of the expression results.

Results

CX11314 and EG4725 Are Genetically and Evolutionarily Divergent from N2:

The genetic divergence among the *C. elegans* strains is highlighted in Figures 4 and 5, where PCA shows the clustering of the strains based on genetic similarities.

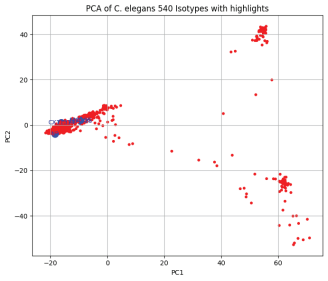

Figure 4: Principal Component Analysis (PCA) depicting the clustering of genetically similar *C. elegans* strains. This clustering demonstrates genetic separation among strains based on SNP variation.

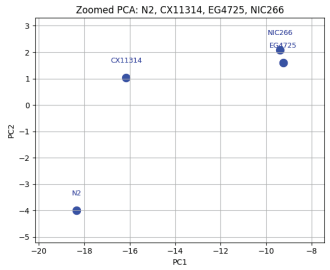

Figure 5: A zoomed-in view depicting the *C. elegans* strains of interest. The zoomed-in view emphasizes the relative genetic distance of CX11314 and EG4725 from N2.

A principal component analysis (PCA) was then utilized to visualize the genetic distance between the 540 different *C. elegans* strains using the SNP data. In both Figures 4 and 5, the data points represent N2, CX11314, and EG4725. Additionally, as the gene expression data for EG4725 was limited,

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NIC266, the strain genetically closest to EG4725, was used as a proxy. The evolutionary relationships among the strains are visualized in Figure 6, providing insights into their genetic distance.

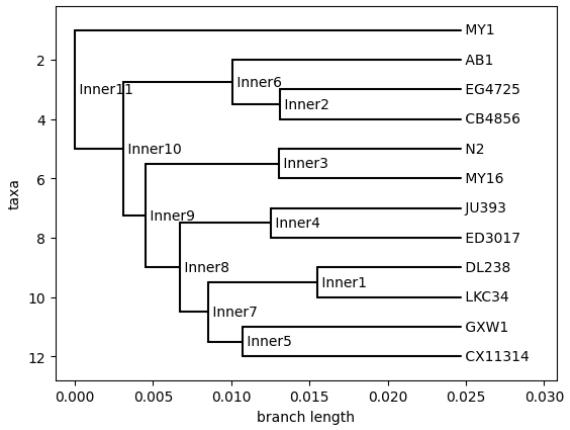

Figure 6: Phylogenetic tree showing evolutionary relationships in different *C. elegans* strains. This tree confirms that CX11314 and EG4725 diverged earlier from the N2 lineage.

The phylogenetic tree based on SNP data further illustrated the evolutionary relationships among the selected strains. Figure 6 shows that CX11314 and EG4725 are both genetically and evolutionarily distinct from N2. For example, the last common ancestor between N2, CX11314, and EG4725 was located at the Inner10 node, suggesting that these strains did not diverge recently.

Purifying and Neutral Selection in Genes:

Table 1: Ratio of non-synonymous to synonymous mutations for respective genes. These results indicate that several mitochondrial genes are evolutionarily conserved under selective pressure.

Gene	dN	dS	dN/dS	Significance
atfs-1	34	41	0.829	Purifying
pink-1	5	5	1	Neutral
drp-1	12	20	0.6	Purifying
pdr-1	15	19	0.789	Purifying
eat-3	5	5	1	Neutral
fzo-1	5	5	1	Neutral

Table 1 presents the dN/dS ratio of the genes of interest, revealing whether the mutations were under purifying selection. The dN/dS ratio measures the rate of non-synonymous mutations over the rate of synonymous mutations, and we used this to determine how genes in strains evolved by seeing which mutations were favored. Using aligned coding sequences from wild *C. elegans* strains, we found that *drp-1*, *pdr-1*, and *atfs-1* were under purifying selection, suggesting that changes in these genes were often deleterious and, in turn, selected for through evolution. Conversely, *pink-1*, *eat-3*, and *fzo-1* each had a dN/dS ratio of 1, indicating that these genes are evolving randomly.

Changes in Protein Structure Due to Missense Variants:

Table 2 summarizes the Variant Effect Predictor (VEP) results for the six mitochondrial genes of interest, showing the proportion of synonymous, missense, and stop-gained variants across CX11314 and EG4725. Table 3 further details the specific amino acid substitutions identified in each gene, highlighting the molecular changes that may affect protein structure and function.

Table 2: VEP results for candidate genes across CX11314 and EG4725. These variant distributions suggest gene-specific differences in evolutionary constraint.

Genes	Synonymous Variants	Missense Variants	Stop Gained
fzo-1	56%	44%	0
eat-3	52%	48%	0
drp-1	75%	25%	0
pink-1	59%	41%	0
atfs-1	55%	45%	0
pdr-1	53%	43%	3%

Table 3: List of specific amino acid substitutions found in the six genes of interest across CX11314 and EG4725 strains. Several substitutions involve changes in polarity or charge, which may alter protein stability and function.

Gene	Number of Variants	Specific Variants
fzo-1	5	1 P->S, 1 R->Q, 1 A->T, 1 V->M, 1 L->I
eat-3	5	1 I->M, 1 A->P, 1 K->R, 1 V->A, 1 A->T
drp-1	12	4 K->R, 4 L->I, 4 T->I
pdr-1	15	3 S->T, 4 E->K, 4 G->D, 2 L->R, 2 F->L
atfs-1	34	7 P->L, 7 Q->R, 7 S->T, 6 V->A, 7 Y->N
pink-1	5	1 E->K, 1 A->S, 1 P->S, 1 V->M, 1 N->D

The VEP and amino acid substitution analyses are summarized in Tables 2 and 3. VEP analysis of the six genes of interest in CX11314 and EG4725 revealed gene-specific synonymous, missense, and stop-gained variants that may influence protein structure or function. For fzo-1, 56% of variants were synonymous, and 44% were missense variants. Conservative substitutions included Valine to Methionine and Leucine to Isoleucine. Other substitutions, such as Proline to Serine, Alanine to Threonine, and Arginine to Glutamine, may alter polarity or charge and therefore could affect protein structure or function.

Secondly, the variations in eat-3 consisted of 52% synonymous variation and 48% missense variation. In the case of eat-3, which consisted of 5 amino acid variations, most of the variations (Isoleucine to Methionine, Lysine to Arginine, and Valine to Alanine) were conservative. However, substitutions that change polarity, such as Threonine (polar) for Alanine (nonpolar), and changes in size, such as Alanine to Proline.

Even though *drp-1* had 12 missense variants, it had the highest synonymous variant percentages at 75%, suggesting that this gene has been evolutionarily conserved. In this case, *drp-1* had 4 of each type of missense variation: Lysine to Arginine, Leucine to Isoleucine, and Threonine to Isoleucine. All these, except Threonine to Isoleucine, are conservative changes that preserve its characteristic.

Unlike the other genes, pdr-1 included 53% synonymous variants, 43% missense variants, and 3% stop-gained variants, suggesting that some variants may potentially lead to truncated protein products with altered functionality. Among the 15 missense substitutions, six substitutions, including Glycine to Aspartic acid and Leucine to Arginine, involved polarity or charge changes.

Interestingly, *atfs-1* had the highest number of missense variants at 34. 45% of the variants were missense, and 53% were synonymous; however, none of the substitutions caused by the missense variants resulted in a change in polarity. Instead, there were 7 substitutions (Glutamine to Arginine) that changed

from a neutral to basic pH; nonetheless, this will still result in an altered structure and function of the protein.

Finally, pink-1 showed 59% synonymous variants and 41% missense variants. Several missense substitutions may still be functionally important because they involve notable biochemical changes, including Glutamic acid to Lysine, Asparagine to Aspartic acid, Alanine to Serine, and Proline to Serine.

DEG Analysis Reveals Statistical Significance of Certain Transcripts:

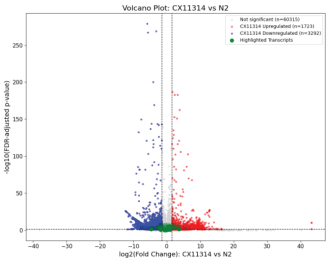

Figure 7: Volcano plots showing differential gene expression in CX11314 and N2. Significant transcripts are highlighted based on adjusted p-values.

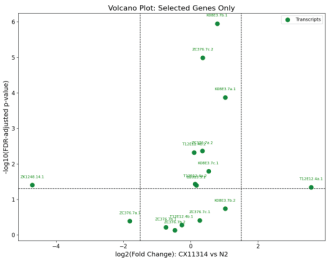

Figure 8: Zoomed-in view of selected mitochondrial genes of interest. The zoomed-in views emphasize mitochondrial genes relevant to quality control.

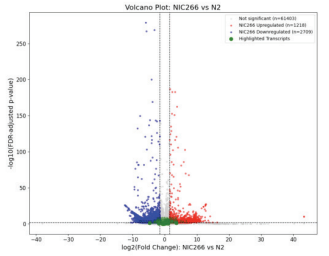

Figure 9: Volcano plots showing differential gene expression (log_2 fold change vs. p-value) between NIC266 and N2. Significant transcripts are highlighted based on adjusted p-values.

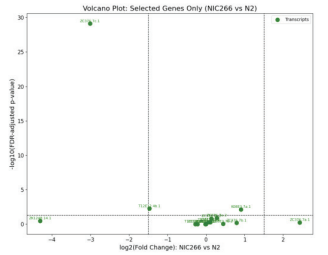

Figure 10: Zoomed-in view of selected mitochondrial genes of interest. The zoomed-in views emphasize mitochondrial genes relevant to quality control.

The DEG results between CX11314 and N2 are shown in Figure 7, while Figure 8 provides a closer view of selected mitochondrial genes. The DEG results for NIC266 and N2 are shown in Figures 9 and 10. Volcano plots were used to visualize differential expression by comparing statistical significance (adjusted p-value) with the magnitude of expression change (log2 fold change). Figure 7 shows that most transcripts corresponding to the genes of interest did not reach statistical significance in CX11314 compared with N2. However, the zoomed-in view in Figure 8 shows that several mitochondrial-related transcripts had observable expression shifts, including ZK1248.14.1 corresponding to fzo-1 and T12E12.4a.1 corresponding to drp-1. Similarly, the NIC266 vs N2 comparison in Figures 9 and 10 showed that only a small subset of transcripts, including ZC276.7c.1 corresponding to atfs-1, T12E12.4b.1 corresponding to drp-1, and K083.7a.1 corresponding to pdr-1, reached statistical significance. Because NIC266 was used as a proxy for EG4725, these results should be interpreted cautiously and confirmed with direct EG4725 expression data in future work.

Higher Expression of pdr-1, atfs-1, and eat-3:

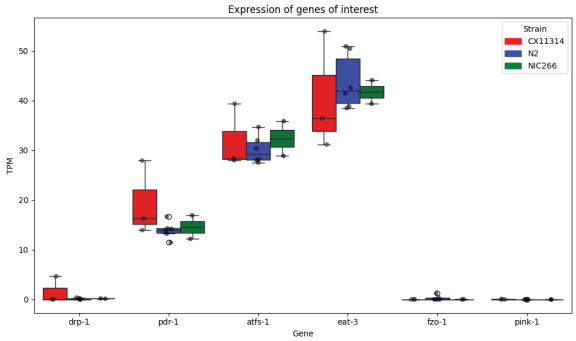

Figure 11: Box plot comparing TPM expression levels of selected mitochondrial genes across N2, CX11314, and NIC266. The figure highlights strain-specific expression patterns, including relatively high pdr-1 expression in CX11314 and high overall eat-3 expression.

The expression levels of the key genes across different strains are compared in Figure 11. The TPM values show that eat-3 had the highest overall expression among the six genes, while pdr-1 showed relatively higher expression in CX11314 than in the other displayed strains. In contrast, fzo-1 and pink-1 showed low expression across the strains. Although not all differences reached statistical significance, the strain-specific patterns suggest that expression variation may still contribute to fine-tuned mitochondrial regulation.

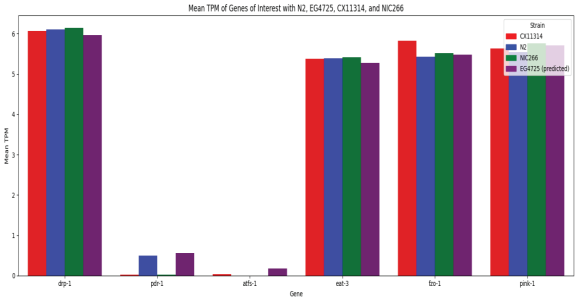

Figure 12: Bar graph comparing mean TPM values of N2, CX11314, NIC266, and predicted EG4725. The alignment between NIC266 and predicted EG4725 supports the use of machine-learning-based prediction as an exploratory approach when direct EG4725 RNA-seq data are unavailable.

The predicted gene expression profile for EG4725 is displayed in Figure 12. The model was trained on SNP and gene expression data from 204 *C. elegans* strains, and a neural network with LOOCV was used to estimate the missing EG4725 expression profile. NIC266 was excluded during validation because it was the genetically closest available proxy for EG4725. Spearman correlation analysis showed a strong prediction confidence (rho = 0.959), supporting the reliability of the predicted profile for exploratory interpretation. However, because these values are predicted rather than directly measured, they should be confirmed through future RNA-seq experiments on EG4725.

■ Discussion

Overall, the combined analyses suggest that natural genetic variation in these six key genes may contribute to enhanced mitochondrial quality control in the wild *C. elegans* strains CX11314 and EG4725. Although many DEG results did not

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reach statistical significance, the observed expression patterns, PCA clustering, phylogenetic divergence, and VEP results together support the possibility that these genes fine-tune mitochondrial regulation under different genetic backgrounds. The missense variants identified in Tables 2 and 3 may alter protein structure or function through changes in polarity, charge, pH, or molecular size. In addition, the predicted EG4725 expression profile showed higher expression of pdr-1 and atfs-1, suggesting a potential link with stronger mitochondrial quality control. These findings indicate that naturally occurring variation in CX11314 and EG4725 may contribute to fitness under environmental pressure and provide candidate targets for future mitochondrial disease research.

Therefore, we propose using CRISPR-Cas9-mediated homology-directed repair (HDR) to swap both promoter and protein-coding regions of selected genes of interest. This method is appropriate because CRISPR-Cas9 genome editing allows precise replacement of genomic regions with donor DNA sequences.[25] Specifically, we propose replacing the N2 promoter and coding regions of pdr-1 and atfs-1 with the corresponding regions from EG4725. This proposal is based on the predicted higher expression of pdr-1 and atfs-1 in EG4725, as shown in Figure 12. Because Figure 12 presents expression on a logarithmic scale, even visually modest differences may represent larger underlying expression differences. These experiments would help determine whether the EG4725 alleles directly contribute to improved mitochondrial quality control.

To appropriately conduct these experiments, we propose using four experimental groups: N2 (control), N2 carrying the EG4725 pdr-1 allele, N2 carrying the EG4725 atfs-1 allele, and EG4725 (baseline). This design would allow the functional effects of individual EG4725 variants to be tested within the N2 background and compared with the native EG4725 strain. It would also reveal whether specific alleles are sufficient to change mitochondrial phenotypes or behavior. Phenotypic and behavioral assays could assess mitochondrial morphology, mitophagy-related activity, ATP and ROS production, stress resistance, lifespan, and overall activity. These experiments would help clarify how genetic variation drives phenotypic differences and may provide a foundation for future therapeutic approaches targeting mitochondrial dysfunction.

From a practical perspective, this study provides a candidate-gene framework that can guide future experimental validation rather than functioning as a final causal proof. The results help prioritize pdr-1, atfs-1, and related mitochondrial quality-control genes for follow-up testing in *C. elegans*. If validated experimentally, these naturally occurring variants may help explain how organisms maintain mitochondrial homeostasis and may inform broader studies of aging, stress tolerance, and mitochondrial disease mechanisms.

Conclusion

This study investigated how natural genetic variation in mitochondrial health-regulating genes contributes to mitochondrial resilience in the *Caenorhabditis elegans* strains CX11314 and EG4725. By integrating variant analysis, gene expression information, and functional interpretation of key mitochondrial pathways, we identified consistent differences between these strains and the reference N2 strain. The results suggest that naturally occurring missense variants and regulatory differences in genes involved in mitochondrial dynamics, mitophagy, and stress response may collectively enhance mitochondrial stability and function. Our findings highlight the importance of studying natural genetic diversity, rather than relying solely on laboratory-induced mutations, and how mitochondrial health is maintained under physiological conditions. The observed associations between genetic variation and improved mitochondrial resilience provide a useful framework for exploring how similar mechanisms may operate in other organisms. This work also demonstrates how combining genetic data with pathway-level interpretation can generate biologically meaningful insights, even in relatively small datasets. While the present study focuses on *C. elegans*, the analytical approach may be extended to other model systems and complex traits related to cellular health and aging. Future studies could further validate these findings through targeted functional experiments and explore the broader implications of natural mitochondrial variation in stress tolerance and disease susceptibility.

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■ Author Contribution Statement

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