Studying the Human Neurodegeneration Gene *PLA2G6* in the *Drosophila* Female Germline

Presented to the S. Daniel Abraham Honors Program

in Partial Fulfillment of the

Requirements for Completion of the Program

Stern College for Women

Yeshiva University

April 28, 2022

Sarah Liberow

Mentor: Dr. Josefa Steinhauer, Biology

Table of Contents

Abstract

Parkinson's disease (PD) is a neurodegenerative disease that has rising incidence around the world. This incredibly difficult and taxing illness is highly studied, and research efforts have pointed to the *PLA2G6/PARK14* gene as a possible genetic component of PD. Previous research in my mentor's lab has shown that *PLA2G6* knockout leads to neurodegeneration in *Drosophila melanogaster* fruit flies, measured by decreasing climbing ability with age. Additionally, they found decreased female fertility in *PLA2G6* knockout mutants and discovered mitochondrial damage in the germline, consistent with other research that has demonstrated PLA2G6 localization to mitochondria and its activity in preventing mitochondrial damage in healthy human cells (Seleznev, Zhao, Zhang, Song, & Ma, 2006). My current research explored whether *PLA2G6* acts directly on germline mitochondria and whether there was a contribution from the somatic tissues. We observed significant mitochondrial damage in the germline upon ubiquitous somatic knockdown of *PLA2G6*, and further questioned which somatic tissues require *PLA2G6* function. Results suggested a strong contribution from the neuronal cells, and preliminary results on the muscle cells and fat bodies require further investigation. Our research will aid in painting a clearer picture of *PLA2G6*'s function in cells and the disease pathology found in those suffering from *PLA2G6*-associated neurodegeneration.

Background

Parkinson's Disease

With an aging population, it is crucial to study neurodegenerative diseases (ND), including Parkinson's disease, as incidence of ND increases with age. According to the Parkinson's Foundation, over 60,000 Americans are diagnosed with PD every year, and the number of total cases is predicted to surpass 1.2 million by 2030 (Marras, et al., 2018). As one of the most common neurodegenerative movement disorders, Parkinson's is primarily diagnosed clinically as movement difficulties such as tremors, rigidity, bradykinesia (slowed movement), postural instability; it also presents with cognitive and psychological issues such as cognitive decline, depression, and anxiety (Balestrino & Schapira, 2020; Armstrong & Okun, 2020). This progressive disease is characterized by the accumulation of Lewy bodies (protein aggregates) composed of α-synuclein proteins in the dopaminergic neurons in the brain, with the consequent lack of dopamine causing neurodegeneration (Olanow & Brundin, 2013).

PLAN Diseases

Past research has shown that while most PD cases are sporadic, patients with PD are twice as likely to have a first-degree relative with the disease, showing a possible genetic component (Marder, et al., 1996). More recent studies suggest that both genetic and environmental causes can contribute to the disease, although to what extent varies between patients (Kalia & Lang, 2015; Singleton, Farrer, & Bonifati, 2013). Studies have associated several genes with PD, the so-called *PARK* genes, including the *PLA2G6/PARK14* gene (Singleton, Farrer, & Bonifati, 2013). Phospholipase A2 Group VI (*PLA2G6*)-associated neurodegeneration, also known as PLAN, is the second most common form among NDs with brain iron accumulation and is an autosomal recessive disorder

(Gregory & Hayflick, 2013; Morgan, et al., 2006). Loss of function mutations in *PLA2G6* cause autosomal recessive dystonia-parkinsonism, as well as other NDs, such as infantile neuroaxonal dystrophy, which develops in childhood (Esfehani, et al., 2021; Paisan-Ruiz, et al., 2008). Polymorphisms in the *PLA2G6* gene also might contribute to sporadic PD.

PLA2G6 Function

The *PLA2G6* gene encodes a phospholipase A2 (PLA2) enzyme that is important for cell membrane remodeling and repair (Lands, 2000; Murakami, et al., 2011). Cell membranes are primarily composed of phospholipids, molecules composed of a polar phosphate group head, two fatty acid tails, and a glycerol backbone (Structural Lipids in Membranes, Chapter 9). The diverse structures of phospholipids in the cell, with varying head groups and fatty acid tails, are vital to cell structure and function (van Meer, Voelker, & Feigenson, 2008). This diversity is accomplished in part by enzymes such as phospholipase A2 (PLA2), which can cleave phospholipids at the *sn-2* position, thus releasing or exchanging a fatty acid tail. The group 6 calcium-independent PLA2 encoded by the *PLA2G6* gene participates in this membrane remodeling and repair (Kita, Shindou, & Shimizu, 2018; Schaloske & Dennis, 2006; Sun, et al., 2021). Membrane repair is crucial to mitochondrial maintenance, and animal models of *PLA2G6*-associated neurodegeneration show mitochondrial abnormalities in neurons (Morgan, et al., 2006). This is consistent with the observed localization of PLA2G6 to mitochondria in healthy cells and with the activity of *PLA2G6* in preventing mitochondrial damage (Kinghorn, et al., 2015; Seleznev, Zhao, Zhang, Song, & Ma, 2006). Thus, it has been proposed that *PLA2G6* prevents neurodegeneration by protecting mitochondria from accumulated membrane damage, consistent with the known role of mitochondrial damage in neurodegeneration (Hewitt & Whitworth, 2017). However, it is unclear how PLA2G6 carries out this function biochemically.

Animal Models

Animals have long been used in research as human disease models, as human physiological functions and anatomical aspects can be similar and can be used to test disease pathologies and therapeutic effects *in vivo*. For example, the mouse model is useful as it shares homology with over 95% of its genes with humans (Barré-Sinoussi & Montagutelli, 2015). However, both mouse models and other common model organisms such as the zebrafish require more staff, equipment, and funding, and they provide a smaller sample size of model animals and have a long lifespan (Mohr, 2018). To better understand the *PLA2G6* gene, our lab utilizes the *Drosophila melanogaster* fruit fly model due to its genetic similarity and evolutionary conservation of human cellular processes; the *Drosophila* genome carries about 60% homology with humans, and over 75% of human disease-causing genes have orthologous genes in *Drosophila* (Mirzoyan, et al., 2019; Ugur, Chen, & Bellen, 2016). Fruit flies are relatively easy to breed and are comparably low maintenance with respect to other animal models (Mohr, 2018). *Drosophila*'s compact genome allows for stable inbred stocks carrying genes of interest that can be crossed to other stocks as needed to result in the desired phenotype (Hales, Korey, Larracuente, & Roberts, 2015). The phenotypes resulting from *Drosophila* mutations can be easily observed over the short lifespan of the fruit fly, and defects can be measured and quantified across large sample sizes, as great amounts of progeny can be bred in a small amount of time (Ashburner, Golic, & Hawley, 2005). Fruit flies—like humans—have five senses, an internal clock, and a complex brain that can control food searches, courting and mating behavior, learning, and fighting (Mohr, 2018). Additionally, due to the functional homology of neurons in the adult *Drosophila* brain to those of humans, *Drosophila* is an ideal model to study NDs like Parkinson's (Hewitt & Whitworth, 2017).

GAL4-UAS System

In order to directly introduce a gene of interest in *Drosophila* to study its effects, the GAL4-UAS system is used. Designed by Andrea Brand and Norbert Perrimon in 1993, the system allows genes to be expressed both rapidly and selectively, with the ability to narrow gene expression to specific tissues or cell types (Brand & Perrimon, 1993). One *P*-element vector contains the yeast transcription factor GAL4 downstream of a *Drosophila* promoter or enhancer; the other *P*-element vector contains an upstream activating sequence (UAS) connected to a gene of interest. The GAL4 element can be under the regulation of a tissue-specific promoter/enhancer that allows for restriction of GAL4 expression to certain tissues. Consequently, GAL4 binds to the UAS sequence and expresses the gene of interest in those tissues alone (Hales, Korey, Larracuente, & Roberts, 2015). Transgenic fly lines containing one construct alone (either the GAL4 driver or the UAS sequence) will not express the gene, so fly lines can easily be crossed and progeny containing both constructs can be selected (Figure 1). My research uses RNA interference (RNAi) to knock down a gene's expression by expressing RNA hairpins using the GAL4 system. These short inverted repeat RNA hairpins are inserted downstream of the UAS and target the gene of interest's messenger RNA and thus do not affect the gene sequence, but rather reduce its expression at either a specific stage in development or in specific cell types (Hales, Korey, Larracuente, & Roberts, 2015).

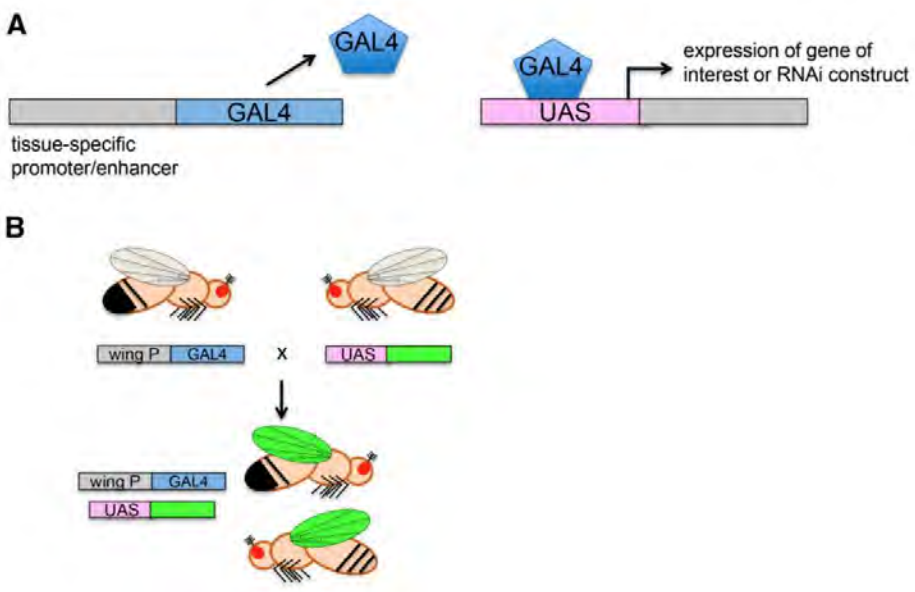

Figure 1 The GAL4-UAS system is used to express a gene of interest. (A) The yeast GAL4 transcription factor is expressed under a tissue or driver and activates the UAS which expresses the gene of interest. (B) Flies containing either the GAL4 or UAS construct are mated to produce progeny containing both constructs. In this example, a wing-specific GAL4 driver is used to express green fluorescent protein in wings only. Photo Retrieved from Hales, Korey, Larracuente, & Roberts, 2015.

Past Results

Mutation of the *Drosophila PLA2G6* ortholog *iPLA2-VIA* causes neurodegenerative effects, similar to those seen in human patients (Mori, et al., 2019; Lin, et al., 2018; Iliadi, Gluscencova, Iliadi, & Boulianne, 2018). In particular, loss of climbing ability is a well-established symptom of neurodegeneration in flies, and a homozygous loss of function mutation $iPLA_2\text{-}VIA^{\Delta 23}$ causes degenerative effects, including age-dependent loss of locomotor ability similar to those seen in human patients. Loss of locomotor ability was phenocopied by RNAi knockdown (KD) in all somatic tissues, in neurons, or in muscle, using the *tub-GAL4*, *elav-GAL4*, and *DJ667-GAL4* drivers, respectively. It was rescued by a wild-type transgene and, surprisingly, also by a mutant transgene in which the catalytic serine was replaced with alanine (Banerjee, et al., 2021). These

interesting results suggest that there is still much unknown about the function and mechanism of *iPLA2-VIA*, including whether its catalytic activity is necessary to protect from neurodegeneration.

In addition, PLA2s have been implicated in fertility. Cytoplasmic PLA2 null mice exhibit reduced fertility in smaller litter sizes compared to healthy controls; the study theorized that this was due to the disruption of the prostaglandin synthesis pathway of which PLA2 is a part (Bonventre, et al., 1997). Another study found that *PLA2G3* is expressed in the epididymal epithelium and *PLA2G3* null mice resulted in sperm malformation due to impaired membrane remodeling (Sato, et al., 2010). In a *Drosophila melanogaster* model, inactivation of orthologous *PLA2G6* (*iPLA2-VIA*) in sterile, *tafazzin*-deficient males restored fertility in double-mutant flies, suggesting that *iPLA2-VIA* plays a role in male fertility (Malhotra, et al., 2009).

Previous research in the lab observed strong expression of *iPLA2-VIA* in both the male and female germline in adult flies; however, male null mutant flies show no effect on fertility (Figure 2A). Nevertheless, female fertility is reduced in $iPLA_2\text{-}VIA^{\Delta 23}$ homozygotes and hemizygotes (shown in reduced progeny and reduced egg-laying). Localization of HA-tagged wild-type *UAS*-iPLA$_2$-VIA-PB transgene was strongest with the mitochondria, but not the Golgi or Endoplasmic Reticulum in adult female germ cells (Figure 2B-D). In aged homozygous mutant females, mitochondria (tagged with mito-YFP) exhibit abnormal aggregation in the germline nurse cells in contrast to the evenly distributed mitochondrial network in control flies. ImageJ analysis of confocal micrographs reveals a highly significant quantitative reduction in germline mitochondrial distribution in *iPLA2-VIA* mutant germlines compared to controls, in females 3 weeks of age and older (Figure 2E-J). Aged mutant females also exhibit reduced JC-1 fluorescence compared to controls, indicating loss of mitochondrial potential. Mitochondrial aggregation and reduced mitochondrial potential are both signs of mitochondrial damage. By 5 weeks of age, *iPLA2-VIA*

mutant germ cells undergo apoptosis, with fragmented nuclei and cleaved caspase-3 staining, which can result from mitochondrial damage (Figure 2L). These defects in female germ cells mirror cytopathologies associated with neurodegeneration, so studying the effects of *PLA2G6* in the female germline may contribute to our understanding of *PLA2G6*-associated neurodegeneration (Banerjee, et al., 2021).

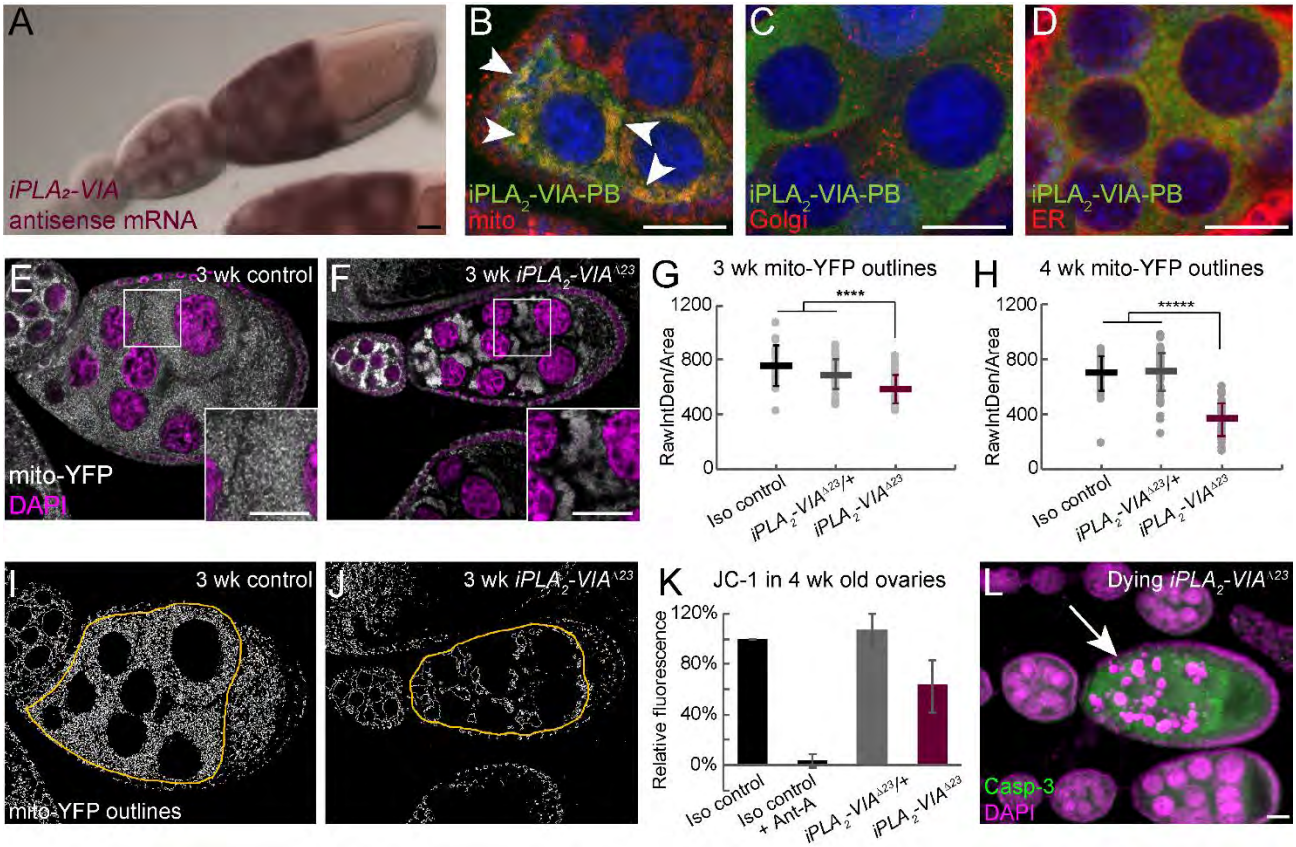

Figure 2 Scale bars: 20 μm (A) in situ hybridization to endogenous *iPLA2-VIA* mRNA (purple) shows strong expression in the female germline. (B-D) In the female germline, HA-tagged wild-type iPLA$_2$-VIA-PB (green) strongly colocalizes with a mitochondrial marker (B, red, Psqh-mito-EYFP, arrowheads) and shows minimal colocalization with Golgi (C, red, anti-Golgin 84) and ER (D, red, anti-Calnexin 99A) markers. (E-F) Mito-YFP labeled mitochondria appear clumpy in germ cells from aged (3 week old) iPLA$_2$-VIA$^{\Delta 23}$ female flies (F) but not controls (E). (G-J) Mito-YFP signal within the area of the germline nurse cells was outlined using ImageJ (white in I-J, yellow delimiting outlines indicate the region of interest, i.e., the nurse cells) and the raw integrated density of the mito-YFP outline signal was used to quantify clumpiness (G-H). (K) Ovaries from 4-week-old iPLA$_2$-VIA$^{\Delta 23}$ females (dark red bar) show reduced JC-1 fluorescence compared to age-matched controls (black, Psqh-mito-EYFP/revertantΔ11; gray, Psqh-mito-EYFP, iPLA$_2$-VIA$^{\Delta 23}$/revertantΔ11) using plate-based fluorimetry. In each experiment, the

red (595 nm) JC-1 fluorescence of the heterozygote or the mutant is expressed as a percentage of the fluorescence of the control, and 3–4 biological replicates are averaged. Error bars are standard deviations. Antimycin A was used as a mitochondrial poison to demonstrate the specificity of the JC-1 signal. (L) By 5 weeks of age, iPLA$_2$-VIA$^{\Delta 23}$ mutants show increased levels of germline apoptosis, marked by cleaved caspase-3 staining (green) and fragmentation of the nurse cell nuclei (DAPI, magenta).

Current Experiment

I hypothesized that *iPLA2-VIA* acts directly on mitochondria in the female germline to prevent damage. This is supported by the strong expression of *iPLA2-VIA* in wild-type female germ cells and iPLA2-VIA protein localization to germline mitochondria. I tested this hypothesis by performing cell-type-specific knockdown using the GAL4-UAS system for tissue-specific genetic manipulation in *Drosophila,* as used for the climbing experiments described above (Caygill & Brand, 2016). I used the fluorescent mito-YFP marker protein to visualize germline mitochondria, with imaging, image processing, and data analysis as performed previously in the lab. Using RNAi knockdown, I tested whether *iPLA2-VIA* is necessary in the germ cells or the soma for germline mitochondrial maintenance. By crossing flies carrying the mito-YFP marker and UAS-*iPLA2-VIA*-RNAi with flies carrying a GAL4 driver for either germline and somatic cells, I created flies with *iPLA2-VIA* knockdown in either germ or somatic cells only. I dissected these flies at various ages and stained their mitochondria. I tested whether loss of *iPLA2-VIA* in either germ or somatic cells only causes mitochondrial aggregation, as was seen in the *iPLA2-VIA* mutant. I compared my knockdown flies to control flies carrying the mito-YFP marker and each GAL4 driver without the *iPLA2-VIA* knockdown. If knockdown in germ cells leads to mitochondrial aggregation, then my hypothesis was correct and iPLA2-VIA acts in the germline directly. However, if knockdown in somatic cells leads to germline mitochondrial aggregation, then iPLA2-VIA functions non-autonomously in the soma to protect germline mitochondria, and the lab will search for the specific tissues involved.

Results

iPLA2-VIA knockdown in germline somatic cells leads to germline mitochondrial aggregation and germ cell death

We hypothesized that due to *iPLA2-VIA*'s strong expression in the germline, mitochondrial localization, and abnormal aggregation due to *iPLA2-VIA*'s absence, *iPLA2-VIA* acted autonomously within the germline to protect mitochondrial integrity. We therefore tested adult female flies with knockdown of *iPLA2-VIA* in the female germline (maternal triple driver *mtd-GAL4*) at 28, 35, and 42 days. Surprisingly we did not find a strong phenocopy of the mitochondrial defect, and significant mitochondrial clumping was observed in comparison to controls only at six weeks (Figure 3A-B). We then tested knockdown of *iPLA2-VIA* in somatic tissues only (*tub-GAL4*), to exclusion of the germline, to examine whether *iPLA2-VIA* functioned non-autonomously and found significant mitochondrial clumping at 28 days and high levels of germ cell death by 35 days (Figure 3D-I).

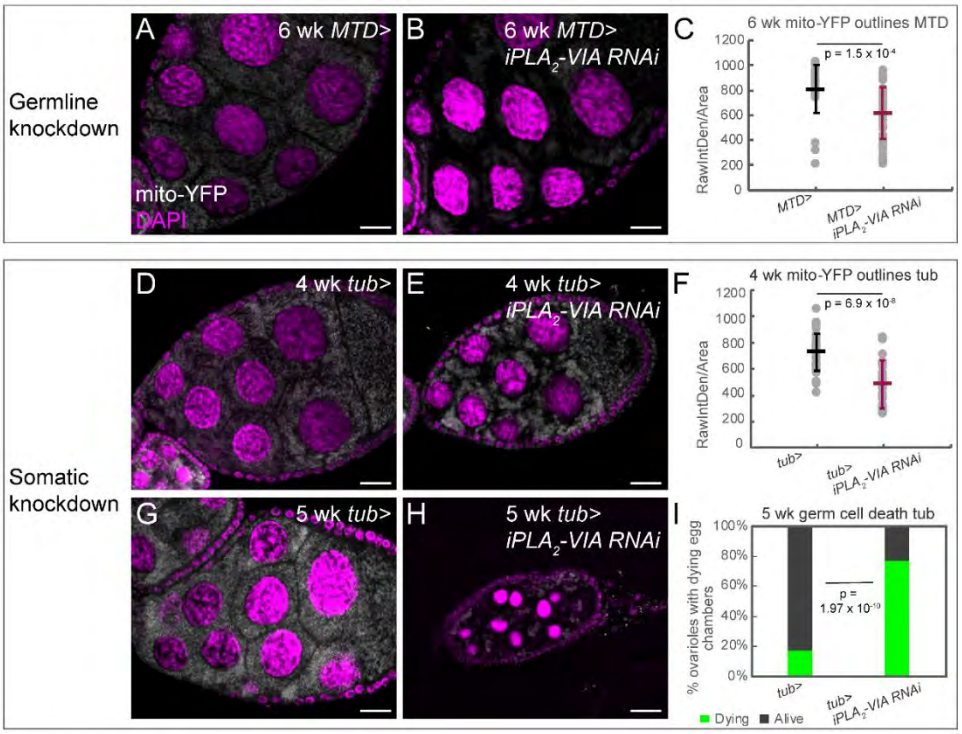

Figure 3 Scale bars: 20 μm. (A-B) Germline knockdown at 42 days: controls n=27, experimental n=23. (C) significant mitochondrial clumping obtained only at six weeks in experimental cohort versus controls. (D-E) Somatic knockdown at 28 days: controls n=30, experimental n=29. (F) Highly significant mitochondrial clumping found at 4 weeks in experimental cohorts versus controls. (G-I) Significantly high levels of germ cell death in KD cohort at 5 weeks versus healthy controls.

iPLA2-VIA knockdown in neuronal tissues leads to germline mitochondrial damage and cell death

To locate the somatic tissue accountable for protecting mitochondrial integrity in the germline, I knocked down *iPLA2-VIA* function in the muscle, fat body, and neurons. Preliminary results of muscle-specific knockdown of *iPLA2-VIA* (*DJ667-GAL4)* suggest mitochondrial aggregation in the germline at 4 weeks of age, but a second experimental set did not replicate this result. Therefore, this experiment requires repetition to definitively understand the results of muscle-specific knockdown. Initial results for knockdown of *iPLA2-VIA* in the fat body also show no effect on germline mitochondria. *iPLA2-VIA* KD in the neuronal tissue (*elav-GAL4*) strongly phenocopied the mutant with extremely significant mitochondrial clumping in comparison to controls at 28 days, with death of older flies (Figure 4).

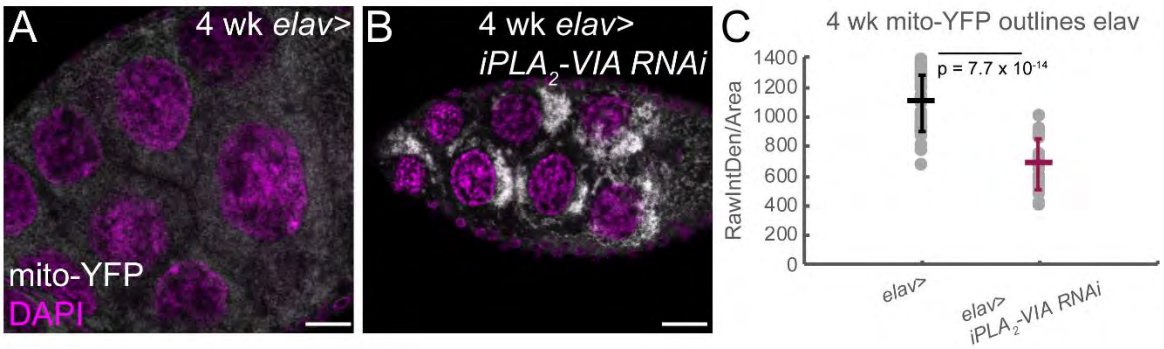

Figure 4 Scale bars: 20 μm. RNAi KD in the neurons at 28 days. (A-B) Controls n=37, experimental n=33. (C) Significant mitochondrial clumping was obtained at four weeks in experimental cohort versus controls.

Discussion

iPLA2-VIA is required in both germline and soma for germline mitochondrial maintenance during aging

iPLA2-VIA knockdown in the germline yielded a weak phenocopy of mitochondrial clumping of the *iPLA2-VIA* mutant in the germline, suggesting only a small autonomous component (Figure 3). However, germline RNAi constructs are known to have difficulty with expression and this result will need to be confirmed with generation of germline clones that are homozygous mutant for *iPLA2-VIA* in an otherwise heterozygous animal to confirm the germline autonomy. The significant amount of abnormal mitochondrial aggregation in the somatic KD suggests a strong non-autonomous component of *iPLA2-VIA* in germline mitochondrial health, pointing to a signaling pathway between one or more somatic tissues and the germline (Figure 3).

Furthermore, in autonomously functioning cells, mitochondrial dysfunction leads to increased sensitivity to reactive oxygen species (ROS) which is implicated in many NDs (Kinghorn, et al., 2015). It is possible that *iPLA2-VIA* protects cells from oxidative stress (and consequently, apoptosis) due to its role in mitochondrial maintenance, and that *iPLA2-VIA* KD flies are vulnerable to programmed cell death due to defective mitochondria. However, this protective mechanism is established only for autonomous cells where mitochondrial abnormalities in a cell can lead to oxidative stress within the same cell. Our data shows that the somatic KD exhibited higher levels of mitochondrial aggregation in the germ cells in comparison to the germline KD and experienced germ cell death by 35 days, whereas the germline KD was not even showing significant clumping at this timepoint. This suggests that *iPLA2-VIA* has a protective effect on the aging germ cells' longevity, but as it is functioning non autonomously, points to something

exciting which is not yet understood—how lack of *iPLA2-VIA* expression in one tissue leads to mitochondrial damage in the other. Thus, *iPLA2-VIA* acts in both germline and somatic tissue to protect the germ cells from mitochondrial damage accumulation and cell death during aging but has unexpected elements that need to be explored further.

***iPLA2-VIA* is required in specific somatic tissues for maintenance of the germline mitochondria**

Initial results did not strongly indicate contribution to germline health from the muscle or fat body tissues, which was surprising as muscle KD produced a strong phenocopy of the climbing defect in *iPLA2-VIA* mutants. We expected the germline mitochondrial aggregation to be phenocopied as well, so the experiment will be repeated. Knockdown of *iPLA2-VIA* in the neuronal tissue yielded highly significant mitochondrial clumping in the germline at four weeks (Figure 4). Loss of *iPLA2-VIA* causes age-related reduced mitochondrial membrane potential and mitochondrial degeneration in *Drosophila* brains, suggesting similar *iPLA2-VIA* function in maintaining mitochondrial activity in both neuronal cells and the germline (Kinghorn, et al., 2015). Because neuronal knockdown of *iPLA2-VIA* leads to mitochondrial aggregation in the germline, we conclude that neuronal maintenance affects female fertility during aging. Additionally, as mentioned above, mitochondrial integrity plays a large role in protecting from cell death. Neuronal KD flies exhibited extremely drastic mitochondrial aggregation in the germline and flies did not survive past the 28-day time point, further supporting the connection between *iPLA2-VIA*'s function as a mechanism for healthy aging of both neurons and germ cells.

Future experiments will seek to ascertain *iPLA2-VIA*'s autonomous versus non-autonomous components by reexamining loss of *iPLA2-VIA* in the germline through mutant clones, and by repeating tissue-specific knockdowns of the neurons, muscle and fat body. *iPLA2-VIA* knockdown

in the somatic ovarian follicle cells also will be tested. Additionally, KD in neuronal subtypes will be performed to see which specific neurons are responsible for maintaining mitochondrial integrity in the germline.

With NDs like Parkinson's on the rise, it is necessary to understand the cellular processes contributing to disease pathology in order to develop treatments for suffering individuals. By building a better picture of how *PLA2G6* affects mitochondrial health in the germline, we can begin to understand how its mutation causes mitochondrial dysfunction and cell death. Due to the similarities in female germ cell mitochondrial abnormalities to the cytopathologies associated with neurodegeneration, studying *iPLA2-VIA* in *Drosophila* may reveal biochemical targets against neurodegenerative disease in human patients and help move the fight against ND forward.

Methods

Crosses

Drosophila were raised on standard media at 23°C, and the following stocks were used to set up the experiments:

RNAi and Control

The *UAS-iPLA2-VIA-RNAi* line is HMS1544. The RNAi construct is inserted on the third chromosome. The chromosome also contains the *mito-YFP* marker for mitochondria. The chromosome is balanced on a balancer chromosome marked with Stubble. The genotype of the stock is: (+; +; (HMS1544,mitoYFP)/TM3Sb).

A control stock carrying mito-YFP alone is used for control experiments: (w; Sco/CyO; mitoYFP).

Germline Knockdown

To knock down *iPLA2-VIA* in female germline, we used a stock called MTD-GAL4 (*maternal triple driver GAL4).* The genotype of this stock is:

(otuGAL4; nosNGT.GAL4; MVD1GAL4/TM3Sb).

F1s collected:

Experimental genotype: (+/otuGAL4; +/nosNGT.GAL4; HMS1544,mitoYFP/MVD1GAL4)

Control genotype: (otuGAL4/yw; nosNGT.GAL4/Sco; MVD1GAL4/mitoYFP)

Both experimental and control flies were selected against stubble.

Somatic Knockdown

To knock down *iPLA2-VIA* in somatic cells, we used the *tub-GAL4* driver. This insertion is homozygous lethal and balanced on a balancer chromosome marked with Curly. The genotype of the stock is (w; tubGAL4/CyO; +).

F1s collected:

Experimental genotype: (+/w; +/tubGAL4; +/HMS1544mitoYFP)

Control genotype: (w/w; tubGAL4/Sco; +/mitoYFP)

Experimental flies were selected against stubble and curly, control flies were selected against curly only.

Tissue Specific Knockdowns

The following GAL4 stocks were used to target specific tissues:

Tissue	**GAL4 line**	**Stock genotype**
Fat body	*ppl-GAL4*	$w;\frac{ppl-GAL4}{ppl-GAL4};\frac{+}{+}$
Neurons	*elav-GAL4*	$\frac{elav-GAL4}{elav-GAL4};\frac{+}{+};\frac{+}{+}$

Muscle	*DJ667-GAL4*	$w; \frac{+}{+}; \frac{DJ667-GAL4}{DJ667-GAL4}$

Fat Body Knockdown F1s Collected:

Experimental genotype: (+/w; +/ppl—GAL4; HMS1544,mitoYFP/+)

Control genotype: (w/w; Sco/ppl—GAL4; mitoYFP/+)

Both experimental and control flies were selected against stubble.

Muscle Knockdown F1s Collected:

Experimental genotype: (+/w; +/+; HMS1544, mitoYFP/ DJ667-GAL4)

Control genotypes: (w/w; +/Sco; DJ667-GAL4/mitoYFP) and

(w/w; +/Cyo; DJ667-GAL4/mitoYFP)

Experimental flies were selected against stubble and all flies were selected for controls.

Neuronal Knockdown F1s Collected:

Experimental genotype: (+/elav—GAL4; +/+; HMS1544,mitoYFP/+)

Control genotype: (w/elav—GAL4; Sco/+; mitoYFP/+)

Both experimental and control flies were selected against stubble.

Tissue Staining and Immunofluorescence

For ovary antibody staining, adult females were mated to males on live yeast paste for 1–2 days before dissections. Ovaries were dissected in PBS and combed open, fixed in 5% formaldehyde for 13 min at room temperature, rinsed in PBX, washed 3–4 times in PBX, and blocked in PBS + 5% normal donkey serum + 1% Triton X-100 before primary antibody incubation. Antibody incubations were performed as above.

Primary antibodies used were rabbit anti-GFP (1:5000, Life Technologies, A6455). Secondary antibodies were Alexa Fluor® 488-AffiniPure Donkey Anti-Rabbit IgG and Cy3-AffiniPure Donkey Anti-Rabbit IgG (1:200, Jackson ImmunoResearch).

Images were captured using a Zeiss LSM510 Confocal (lens: 40x oil/1.30 NA).

Mito-YFP Quantification

Ovaries were dissected and stained with anti-GFP antibodies as described above. Every stage 8 or 9 egg chamber from each sample was photographed using identical confocal settings and 40x oil objective. After drawing an ROI around the nurse cell area and removing background signal with the same threshold, each image was converted to binary in ImageJ. Each binary image was converted to outlines, and the raw integrated density was measured for the ROI.

Acknowledgements

Thank you Dr. Steinhauer for taking me into your lab and teaching me so much. I am so grateful for the hours you have spent training me, the opportunities you have given me, and for making me a better scientist. The past two years in your lab have been the most enriching experience. Thank you to my lab partner, Eliezer Heller, for your part in all our experiments. I could not have done it without you! Thank you to Jeremy Purow for your assistance on some experiments and Dr. Cynthia Wachtell, for giving me the incredible opportunity of being in the S. Daniel Abraham Honors Program at Stern and being able to write this thesis.

Bibliography

Armstrong, M. J., & Okun, M. S. (2020). Diagnosis and Treatment of Parkinson Disease: A Review. *JAMA, 323*(6), 548–560. doi:10.1001/jama.2019.22360

Ashburner, M., Golic, K. G., & Hawley, R. S. (2005). *Drosophila: a laboratory handbook.* Cold Spring Harbor Laboratory Press.

Balestrino, R., & Schapira, A. (2020). Parkinson disease. *European journal of neurology, 27*(1), 27-42. doi:10.1111/ene.14108

Banerjee, S. J., Schonbrun, A., Eizadshenass, S., Benji, S., Cantor, Y. T., Eliach, L., . . . Steinhauer, J. (2021). iPLA2-VIA is required for healthy aging of neurons, muscle, and the female germline in Drosophila melanogaster. *PLoS ONE, 16*(9), 1-26. doi:10.1371/journal.pone.0256738

Barré-Sinoussi, F., & Montagutelli, X. (2015). Animal models are essential to biological research: issues and perspectives. *Future Science OA, 1*(4). doi:10.4155/fso.15.63

Bonventre, J. V., Huang, Z., Taheri, M. R., O'Leary, E., Li, E., Moskowitz, M. A., & Sapirstein, A. (1997). Reduced fertility and postischaemic brain injury in mice deficient in cytosolic phospholipase A2. *Nature, 390*, 622-625. doi:10.1038/37635

Brand, A., & Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development, 118*(2), 401-415. doi:10.1242/dev.118.2.401

Caygill, E. E., & Brand, A. H. (2016). The gal4 system: A versatile system for the manipulation and analysis of gene expression. *Methods in Molecular Biology*, 33-52. doi:10.1007/978-1-4939-6371-3_2

Esfehani, R., Eslahi, A., Toosi, M., Sadr-Nabavi, A., Kerachian, M., Mohajeri, M., . . . Mojarrad, M. (2021). PLA2G6 gene mutation and infantile neuroaxonal degeneration; report of three cases from Iran. *Iranian Journal of Basic Medical Sciences, 24*(9), 1190-1195. doi:10.22038/ijbms.2021.55082.12340

Gregory, A., & Hayflick, S. (2013). Neurodegeneration with Brain Iron Accumulation Disorders Overview. In M. P. Adam (Eds.) et. al., GeneReviews®. University of Washington, Seattle.

Hales, K. G., Korey, C. A., Larracuente, A. M., & Roberts, D. M. (2015). Drosophila, Genetics on the Fly: A Primer on the Drosophila Model System. *Genetics, 201*(3), 815-842. doi:10.1534/genetics.115.183392

Hewitt, V. L., & Whitworth, A. J. (2017). Mechanisms of Parkinson's Disease: Lessons from Drosophila. *Current Topics in Developmental Biology, 121*, 173-200. doi:10.1016/bs.ctdb.2016.07.005

Iliadi, K. G., Gluscencova, O. B., Iliadi, N., & Boulianne, G. L. (2018). Mutations in the Drosophila homolog of human PLA2G6 give rise to age-dependent loss of psychomotor activity and neurodegeneration. *Sci Rep, 8*(1). doi:10.1038/s41598-018-21343-8

Kalia, o. V., & Lang, A. E. (2015). Parkinson's disease. *The Lancet, 386*(9996), 896-912. doi:10.1016/S0140-6736(14)61393-3

Kinghorn, K. J., Castillo-Quan, J. I., Bartolome, F., Angelova, P. R., Li, L., Pope, S., . . . Li, L. (2015). Loss of PLA2G6 leads to elevated mitochondrial lipid peroxidation and mitochondrial dysfunction. *Brain: A Journal of Neurology, 138*(7), 1801-1816.

Kita, Y., Shindou, H., & Shimizu, T. (2018). Cytosolic phospholipase A2 and lysophospholipid acyltransferases. *Biochim. Biophys. Acta (BBA)—Mol. Cell Biol. Lipids, 1864*, 838-845.

Lands, W. E. (2000). Stories about acyl chains. *Biochimica Et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 1483*(1), 1-14. doi:10.1016/s1388-1981(99)00177-8

Lin, G., Lee, P.-T., Chen, K., Mao, D., Tan, K. L., Zuo, Z., . . . H. J. (2018). Phospholipase PLA2G6, a parkinsonism-associated gene, affects vps26 and vps35, retromer function, and ceramide levels, similar to α-synuclein gain. *Cell Metabolism, 28*(4). doi:10.1016/j.cmet.2018.05.019

Malhotra, A., Edelman-Novemsky, I., Xu, Y., Plesken, H., Ma, J., Schlame, M., . . . Sabatini, D. D. (2009). Role of Calcium-Independent Phospholipase A_2 in the Pathogenesis of Barth Syndrome. *roceedings of the National Academy of Sciences of the United States of America, 106*(7), 2337-2341. doi:10.1073/pnas.0811224106

Marder, K., Tang, M.-X., Mejia, H., Alfaro, B., Cote, L., Louis, E., & Groves, J. (1996). Risk of parkinson's disease among first-degree relatives. *Neurology, 47*(1), 155-160. doi:10.1212/wnl.47.1.155

Marras, C., Beck, J. C., Bower, J. H., Roberts, E., Ritz, B., & Ross, G. W. (2018). Prevalence of Parkinson’s disease across North America. *npj Parkinson's Disease, 4*(1). doi:10.1038/s41531-018-0058-0

Mirzoyan, Z., Sollazzo, M., Allocca, M., Valenza, A. M., Grifoni, D., & Bellosta, P. (2019). Drosophila melanogaster: A model organism to study cancer. *Frontiers in Genetics, 10*. doi:10.3389/fgene.2019.00051

Mohr, S. E. (2018). *First in fly: Drosophila research and Biological Discovery.* Harvard University Press.

Morgan, N. V., Westaway, S. K., Morton, J. E., Gregory, A., Gissen, P., Sonek, S., . . . Hayflick, S. (2006). PLA2G6, encoding a phospholipase A2, is mutated in neurodegenerative disorders with high brain iron. *Nature genetics, 38*(7), 752-754. doi:10.1038/ng1826

Mori, A., Hatano, T., Inoshita, T., Shiba-Fukushima, K., Koinuma, T., Meng, H., . . . Takahashi, Y. (2019). Parkinson's disease-associated IPLA2-via/PLA2G6 regulates neuronal functions and α-synuclein stability through membrane remodeling. *Proceedings of the National Academy of Sciences, 116*(41), 20689-20699. doi:10.1073/pnas.1902958116

Murakami, M., Taketomi, Y., Miki, Y., Sato, H., Hirabayashi, T., & Yamamoto, K. (2011). Recent progress in phospholipase a2 research: From cells to animals to humans. *Progress in Lipid Research, 50*(2), 152-192.

Olanow, C. W., & Brundin, P. (2013). Parkinson's Disease and Alpha Synuclein: Is Parkinson's Disease a Prion-Like Disorder? *Movement Disorders, 28*(1), 31-40. doi:10.1002/mds.25373

Paisan-Ruiz, C., Bhatia, K. P., Li, A., Hernandez, D., Davis, M., & Wood, N. W. (2008). Characterization of PLA2G6 as a locus for dystonia-parkinsonism. *Annals of Neurology, 65*(1), 19-23. doi:10.1002/ana.21415

Sato, H., Taketomi, Y., Isogai, Y., Miki, Y., Yamamoto, K., Masuda, S., . . . Murakami, M. (2010). Group III secreted phospholipase [A.sub.2] regulates epididymal sperm maturation and fertility in mice. *Journal of Clinical Investigation, 120*(5), 1400. doi:10.1172/JCI40493

Schaloske, R., & Dennis, E. (2006). The phospholipase A2 superfamily and its group numbering system. *Biochim. Biophys. Acta (BBA)—Mol. Cell Biol. Lipids, 1761*, 1246.

Seleznev, K., Zhao, C., Zhang, X. H., Song, K., & Ma, Z. A. (2006). Calcium-independent phospholipase A2 localizes in and protects mitochondria during apoptotic induction by Staurosporine. *Journal of Biological Chemistry, 281*(31), 22275-22288. doi:10.1074/jbc.m604330200

Singleton, A. B., Farrer, M. J., & Bonifati, V. (2013). The genetics of parkinson's disease: Progress and therapeutic implications. *Movement Disorders, 28*(1), 14-23. doi:10.1002/mds.25249

Sun, G. Y., Geng, X., Teng, T., Yang, B., Appenteng, M. K., Greenlief, C. M., & & Lee, J. C. (2021). Dynamic Role of Phospholipases A2 in Health and Diseases in the Central Nervous System. *Cells, 10*(11). doi:10.3390/cells10112963

Ugur, B., Chen, K., & Bellen, H. J. (2016). Drosophila tools and assays for the study of human diseases. *Dis. Model. Mech, 9*, 235-244. doi:10.1242/dmm.023762

van Meer, G., Voelker, D. R., & Feigenson, G. W. (2008). Membrane lipids: where they are and how they behave. *Nature Reviews Molecular Cell Biology, 9*(2), 112-124. doi:10.1038/nrm2330