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A Composite Review of the Proposed Molecular Mechanisms and Genetic Components Underlying Parkinson's Disease

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A Composite Review of the Proposed Molecular Mechanisms and Genetic Components Underlying Parkinson's Disease

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I. ABSTRACT

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the progressive death of dopaminergic neurons present in the substantia nigra. The clinical presentation of PD includes tremors, slowed movement (bradykinesia), muscle and limb rigidity, and difficulty with walking and balancing. While many environmental factors can affect the onset and progression of the disease, genetic mutations have a large influence. Of the identified PD-linked genetic mutations, mutations in the leucine-rich repeat kinase 2 (LRRK2) are one of the most common genetic causes of PD. Located in endosomes, LRRK2 has been shown to play a role in the sorting and endocytosis of synaptic vesicles, a process that is largely mediated by the retromer complex. Mutations in Vps35, a core component of the retromer cargo-recognition complex, have also been identified as a significant cause of late-onset autosomal dominant familial PD. While the exact molecular mechanisms by which LRRK2 and Vps35 mutations induce PD remain largely unknown, their influence on several cellular processes, including vesicular trafficking and breakdown, and endosomal sorting and recycling, strongly implicate the retromer and autophagy in PD pathology. Recent findings that transgenic expression of Vps35 is able to rescue the PD-related phenotypes caused by LRRK2 mutant forms provide further insight into the interplay of these genes in the context of PD and point to these -genes as potential therapeutic targets. This review outlines the current studies involving these genetic mutations and their interactions with various cellular processes and pathways so as to gain a better understanding of the molecular

mechanisms underlying PD pathology for the ultimate purpose of developing safe and effective treatments for PD.

II. BACKGROUND

a) The growing need for effective treatments for PD

Parkinson's disease (PD) is one of the most common neurodegenerative disorders, second only to Alzheimer's disease. Affecting almost 700,000 individuals over the age of 45 in the United States in 2010, PD is expected to continue to increase in prevalence to affect more than one million people in the U.S. by 2030 (Marras et al., 2018). With an average onset around age 55, PD is a progressive disease and demonstrates markedly worse symptoms at its later stages. Due to the loss of dopaminergic neurons in the substantia nigra and the subsequent decrease in dopamine signaling occurring in the basal ganglia, PD is most commonly recognized through an affected individual's loss of motor function. This deterioration of motor function clinically presents as bradykinesia, rigidity in gait and limb movement, postural instability, as well as tremors (Brazier, 2018). Further symptoms can include hyposmia, constipation, mood disorders, sleep disorders, cognitive defects, and dementia which only serve to lower the quality of life for affected individuals (Ascherio & Swarzschild, 2016, Rahman & Morrison, 2019). Because the symptoms and complications of PD are severe and its burden on society is expected to grow as the average lifespan has increased, a larger value has been placed on research focusing on understanding the cellular and molecular processes underlying PD neurodegeneration so as to identify biological targets and develop therapeutic treatments.

b) Drosophila melanogaster as an important animal model for studying PD

Due to limitations of human genetic studies, animal models are important for studying the function of genes and proteins involved in cellular pathways implicated in neurodegenerative disorders. While many model organisms, such as yeast, rats, *C. elegans*, and zebrafish, can provide great insight into the molecular mechanisms underlying PD, many fall short in meeting all of the necessary criteria to carry out a robust and effective study of human PD. The criteria for transgenic models include observable behavioral and physiological defects, a clear pattern of inheritance, a well-defined and easily-analyzed nervous system, and the conservation of cellular pathways (Hirth, 2010). *Drosophila melanogaster*, also known as the common fruit fly, are an especially powerful animal model for studying Parkinson's disease as they are not only easily available, rapidly reproducing, and have short life-spans but also stand alone in their fulfillment of these criteria for studying human neurodegeneration. Transgenic *Drosophila* models with PD-linked genetic mutations, unlike rat models which fail to manifest the cardinal pathological features of PD seen in humans, consistently reproduce dopaminergic neuron death and demonstrate locomotor defects in complicated behaviors, such as climbing and walking (Linhart et al., 2014, Xiong & Yu, 2018). Further, many of the fundamental cellular processes, genes, and signaling pathways are conserved in both *Drosophila* and humans, as many of the genes associated with familial PD have at least one fly homolog (Muñoz-Soriano & Paricio, 2011). This stands in contrast to other proposed model organisms, such as *C. elegans*, which have far fewer gene homologs in mammals and lack many biologically and physiologically relevant systems seen in

both flies and humans. *Drosophila* also possess a wide array of genetic tools including transgenic methods for gene manipulation, systems for controlled ectopic expression of certain genes, and balancer chromosomes. As the *Drosophila* genome is encoded on only four chromosome pairs, balancer chromosomes allow not only allow for inclusion of visible molecular markers but also for the suppression of recombination to effectively follow mutations across many generations (Cauchi & van den Huevel, 2006). One of the most interesting features of the fruit fly, however, is its compound eye. Composed of nearly 800 ommatidia, each of which contain 8 photoreceptor neurons, the *Drosophila* eye is extremely useful for studying neurodegenerative phenotypes as its organized and symmetrical layout of photoreceptors allows for easy quantification of neurodegeneration. In analyzing the eye in flies expressing a PD-linked mutation, the level of pigmentation loss, bristle organization, and appearance of black lesions can provide information about the neurodegenerative phenotype (Mishra & Knust, 2013, Marcogliese et al., 2017). The eye is also tolerant to genetic disruption of biological processes yet vital to fly survival (Sang & Jackson, 2005; Cauchi & van den Huevel, 2006).

In making a fly model to study PD, the most common approaches are to ectopically express a human disease gene (either in wild-type of mutant form) or to generate a loss-of-function mutation in the *Drosophila* homolog. Expressing a human gene through the use of a UAS-Gal4 system can yield insight on its function properties and its interactions with other *Drosophila* genes and proteins. Conversely, inducing a loss-of-function mutation through UAS-Gal4 mediated RNA interference

can provide a better understanding of the role of the fly homolog, thereby allowing for predictions about pathogenic pathways underlying the disease.

c) Autophagy dysfunction is a hallmark of neurodegenerative disorders

Autophagy is an essential cellular process by which cytosolic components in the cell, especially protein aggregates or damaged organelles, are degraded and recycled by lysosomes. Initially discovered in yeast as a physiological response to starvation, autophagy is induced in response to conditions of cellular stress, including nutrient deprivation, oxidative stress, and the presence of abnormal cellular components. New evidence further suggests a neuroprotective role for autophagy through its prevention of the accumulation of harmful products in the brain (Lynch-Day et al., 2012, Papinski et al., 2014). As it is important for the maintenance of homeostatic conditions and is involved in a wide range of physiological functions, dysregulation of autophagy is strongly implicated in the pathophysiology of neurodegenerative disorders. This is supported by evidence demonstrating that autophagy dysfunction leads to the accumulation of abnormal and potentially toxic proteins, a hallmark condition commonly observed in the brains of PD patients (Mizushima & Komatsu, 2011).

There are three forms of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy. The most common type of autophagy in the cell is macroautophagy, which will hereafter be solely referred to as autophagy. The process of autophagy, as outlined in Figure 1, begins when an isolation membrane, or phagophore, sequesters a portion of the cytoplasm–which commonly includes organelles–to form a double-membrane autophagosome at a site close to the vacuolar membrane, known as the preautophagosomal structure (PAS) (Mizushima, 2007).

This structure then fuses with a lysosome to become an autophagolysosome and breaks down the materials it contains.

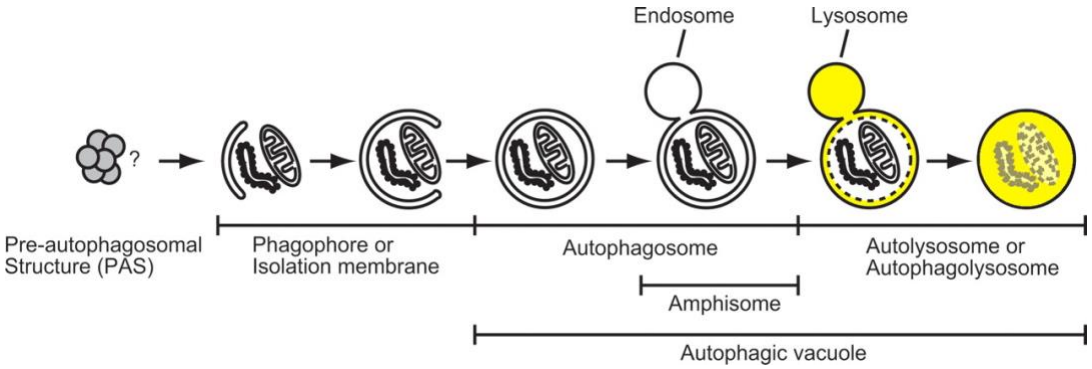

Figure 1. A brief outline of the basic steps of macroautophagy, wherein a phagophore engulfs its cargo to become an autophagosome–whose maturation is mediated by multiple Atg proteins as well as vacuolar protein sorting (VPS) complexes in the PAS–until it fuses with a lysosome to form an autophagolysosome, which thereafter carries out cargo degradation (Figure courtesy of Mizushima, 2007)

The process of autophagy is dependent on specialized autophagy-related (Atg) genes and the proteins they encode. While each of these proteins are necessary for different aspects of autophagy, Atg9, the sole multipass transmembrane membrane, is especially important, as it travels between the site of phagophore nucleation and autophagosome formation and is thought to transport the membrane needed for autophagosome formation (Lynch-Day et al., 2012). The trafficking of Atg9 through the process of endosomal recycling is therefore essential for autophagy. It further colocalizes with the largest subunit, Vps35, of the retromer complex (Zavodszky et al., 2014). The retromer, a protein complex, is crucial for the endosomal sorting machinery that transports and recycles transmembrane receptors from endosomes to the trans-Golgi network (TGN). Atg9 also colocalizes with the WASH complex, an actin-regulatory network of proteins that, along with the retromer at the membrane,

plays a role in endosomal trafficking and recycling (Zavodszky et al., 2014). These association between Atg9 and Vps35 of the retromer complex and the WASH complex demonstrates a functional relationship between the retromer and autophagy– a connection important for understanding PD pathology.

d) The genetic association between the retromer and PD

The retromer was first implicated in the pathology of PD with the discovery of a point mutation to the Vps35 subunit of the retromer. This specific mutation of an aspartate to asparagine at residue 620 (D620N) was identified through exome sequencing in individuals with PD and remains one of the only confirmed pathogenic Vps35 mutations (Vilariño-Güell et al., 2011, Williams et al., 2017). While the exact mechanism by which this Vps35 (D620N) mutation induces neurodegeneration remains unknown, the identification of this mutation in a number of individuals with PD has demonstrated the retromer's pathogenic role in PD development.

The retromer complex, first discovered in yeast, is composed of a cargo-recognition trimer, made up of the proteins Vps35, Vps26, and Vps29, and a membrane-associated dimer of sorting nexin (SNX) proteins Snx1, Snx2, Snx5, and Snx6, in various combinations (Fig 2) (Swarbrick et al., 2011). Responsible for recycling and trafficking cargo molecules and transmembrane proteins from endosomes to the trans-Golgi network (TGN), the retromer complex is an integral part of the endosomal protein sorting system (Maruzs et al., 2015). Because it mediates the recycling of receptors that are involved in the transportation of hydrolytic enzymes to lysosomes, the retromer also plays a part in the early stages of autophagy and endocytosis (Maruzs et al., 2015).

The cargo-recognition complex (CRC) trimer (also referred to as the cargo-selective complex, or CSC), composed of Vps26, Vps29, and Vps35, is responsible for recognizing and binding to trafficking receptors (Williams et al., 2017). Vps35 is the largest protein in the CRC trimer and functions as the scaffold for which Vps29

can bind to its C-terminal end and Vps26 can bind to its N-terminal end (Swarbrick et al., 2011, Williams et al., 2017).

The SNX-BAR dimer, made up of sorting nexin proteins Snx1 or Snx2 and Snx5 or Snx6, induces membrane remodulation and facilitates the stabilization of endosomal tubules. The sorting nexin proteins associated with the retromer complex are characterized by a phox homology (PX) domain, with a high affinity for binding phosphatidylinositol phosphate membrane lipids, and a Bin/Amphiphysin/Rvs (BAR) domain, which functions in recruitment to the membrane and stabilization of tubulation (Collins, 2008).

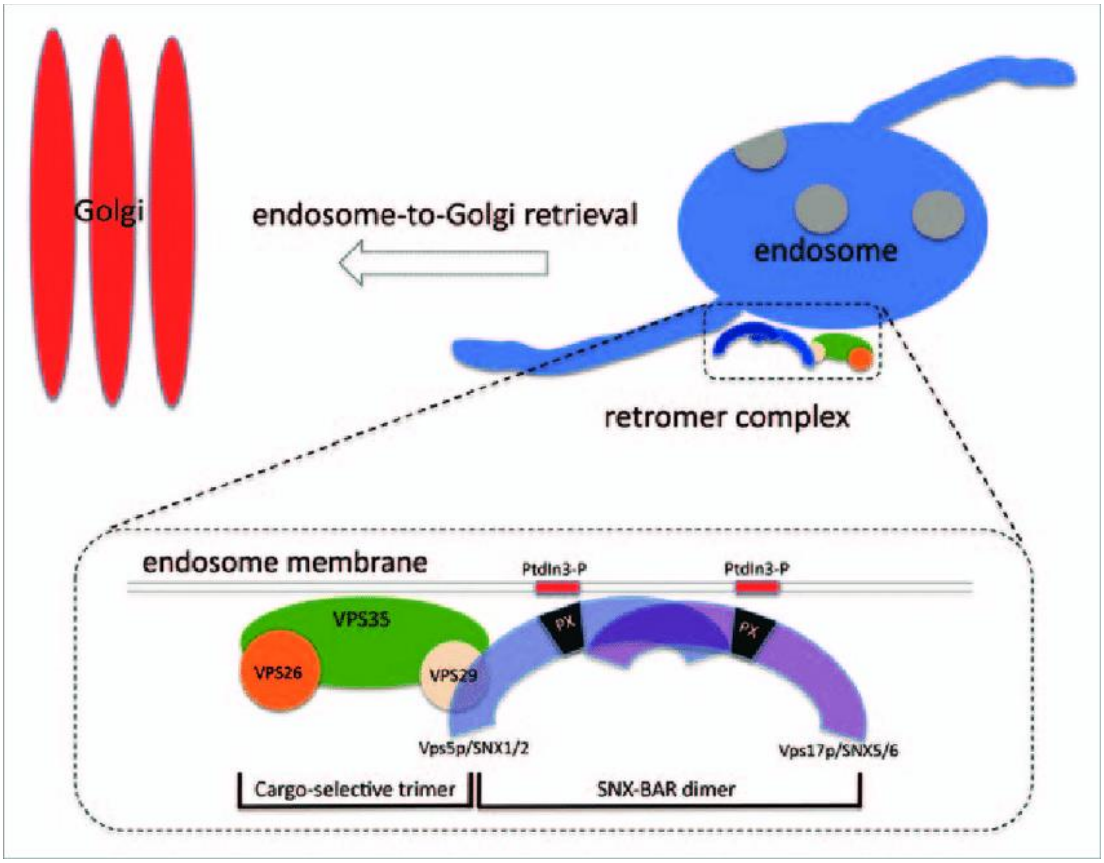

Figure 2. A schematic representation of the retromer complex. Made up of Vps26, Vps29, and Vps35, the cargo-recognition complex (CRC)–or cargo-selective

trimer, as its termed here–interacts with the Snx-BAR dimer largely through the cooperation of Vps35 and Vps29. Vps35 plays an important role in recruitment of the CRC to the endosomal membrane while PI3P–or Ptdln3-P, in this figure–functions as the binding site for the PX domain of the Snx-BAR dimer, allowing for separate recruitment to the membrane. Together making up the retromer complex, the CRC and the Snx-BAR dimer allow for endosome-to-Golgi retrieval of cargo molecules and other proteins (Figure courtesy of Harbour & Seaman, 2011).

To transport cargo, the CRC must first be recruited to the endosomal membrane. It has been shown that Rab7a and Snx3 are required for recruitment of the cargo-recognition complex, as the loss of either causes a displacement of the CRC (Vardarajan et al., 2012). It has further been shown that Snx3 interacts specifically with the Vps35 subunit of the CRC, suggesting that Vps35 is integral for correct recruitment of the CRC to the endosomal membrane and, therefore, for proper cargo trafficking (Seaman, 2012). The Snx-BAR dimer is separately recruited to the membrane of early endosomes and is able to interact with the CRC through its PX domain, which binds to phosphatidylinositol 3-phosphate (PI3P) present on the membrane (Williams et al., 2017). Produced by the phosphoinositide 3-kinase (PI3K), Vps34, PI3P is able to recruit Rab7a and Snx proteins. As the connection between the CRC and Snx-BAR dimer is not very strong, Rab7a is important in strengthening their association with the endosomal membrane.

Another important action of the retromer is its interaction with the Wiskott-Aldrich Syndrome Protein and SCAR Homolog (WASH) complex. Composed of Wash1, Fam21, CCDC53, KIAA1033/SWIP, and strumpellin, the WASH complex plays a role in endosomal sorting, as it is a nucleation-promoting factor and regulates the generation of actin filaments and networks (Seaman et al., 2013, Wang et al., 2014). Shown in Figure 3, the interaction of Vps35 of the retromer complex with the

unstructured tail domain of Fam21 of the WASH complex allows for recruitment of the WASH complex to the endosomal membrane so as to function in endosome-to-cell surface retrieval and trafficking of cellular cargo and receptors. At the membrane, the WASH complex promotes the formation of actin domains which act to restrict retromer cargo proteins and thereby concentrate cargo, such as the cation-independent mannose 6-phosphate receptor (CIMPR), in specific areas for endosome-to-TGN retrieval (Seaman, 2012, Williams et al., 2017). The WASH complex can further interact with Snx27 and the retromer to regulate the endosome-to-plasma membrane transport of ß2-adrenergic receptor (ß2AR), which mediates smooth muscle relaxation, and the glucose transport 1 (GLUT1), which facilities glucose transport to the brain (Williams et al., 2017).

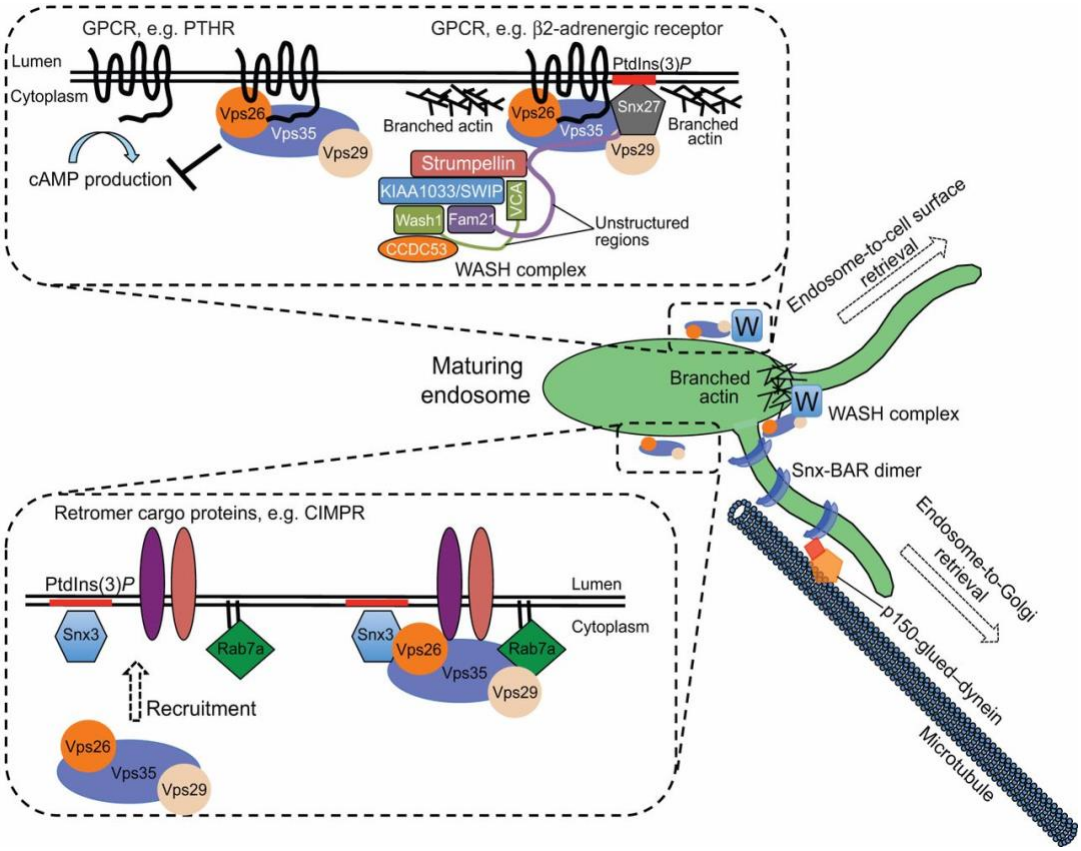

Figure 3. A schematic representation of the interaction of the retromer complex and WASH complex and their role in endosomal sorting. The WASH complex, responsible for regulating actin filament branching, associates with Vps35 of the cargo-recognition complex through the 'tail' of Fam21. The CRC, WASH complex, and Snx27 facilitate the endosome-to-cell surface retrieval of ß2-adranergic receptor. The WASH complex also plays a role in the endosome-to-Golgi retrieval of CIMPR, a retromer cargo protein (Figure courtesy of Seaman, 2012).

e) Leucine-rich repeat kinase 2 (LRRK2) mutations induce PD

Mutations to the leucine-rich repeat kinase 2 (LRRK2) gene are the most common cause of late-onset autosomal dominant PD. Identified LRRK2 mutations, including N1437H, R1441C/G/H, Y1699C, G2019S, and I2020T, are responsible for more cases of familial PD than any other implicated genes (Williams et al., 2017). The mechanisms by which these mutations induce PD pathology, however, remains unclear.

LRRK2 is a large (2527 amino acid) multi-domain protein characterized by its carboxy-terminal of Ras of complex (COR) sequence which links its Ras of complex (Roc) G-domain and kinase domains. Due to its distinct domains, LRRK2 is able to catalyze phosphorylation through its kinase domain and function in GTP-GDP hydrolysis through its ROC-GTPase domain (Li et al., 2014). While many of its roles remain unknown, LRRK2 is expressed in most cells, pointing to its involvement in a variety of basic cellular functions. Further studies have implicated LRRK2 in endolysosomal trafficking, the transport and sorting of proteins, and synaptic vesicle trafficking (Linhart et al., 2014). This was supported by evidence that LRRK2 mutations cause defects in both lysosomal protein degradation and macroautophagy (MacLeod et al., 2013). Alegre-Abarrategui et al. procured direct evidence for a functional relationship of LRRK2 and autophagy by demonstrating that the LRRK2 mutation caused accumulation of autophagic vesicles, a hallmark pathological feature of neurodegenerative disorders, especially PD (2009). The knowledge that both Vps35 and LRRK2 mutations recapitulate key pathological features of neurodegenerative disorders, including the impairment of endosomal cargo

trafficking and sorting as well as the disruption of autophagy, lends itself to the idea that Vps35 and LRRK2 may operate in a common pathway and interact to induce PD pathology.

III. METHODS

Drosophila melanogaster models are typically used to study the effects of the Vps35 (D620N) and LRRK2 (I2020T) mutations and their interactions in causing PD symptoms. While many genetic screens can allow for identification of influential genetic mutations as well as components in pathways of interest, phenotypic analyses are just as important. A climbing assay and eye phenotype analysis can provide information about the presence of key pathological features of PD.

a) Climbing Assay

A reliable and cost-effective system, the climbing assay is used to analyze the locomotor defects of the PD-linked mutant flies. This climbing assay is effective as it capitalizes on negative geotaxis, or the innate escape response of *Drosophila* to ascend the walls of a cylinder after being forced to the bottom.

The day before the climbing assay, 10 female flies are collected using CO_2 anesthetization methods under a microscope and placed in a 3.8 cm x 10 cm collection vial containing a cornmeal food mixture. Left in an incubator kept at 29ºC overnight, the flies were then transferred to an empty collection vial that had a horizontal line drawn on it 8 cm from the bottom and which was closed off with a cotton ball to prevent flies from escaping. The vial was then tapped three times on the table surface so as to displace the flies to the bottom of the vial. The number of flies that crossed the horizontal line after 10 seconds and then 20 seconds was recorded and filmed with a camera placed about 10 inches away from the vial. This

climbing assay was performed on wild-type flies without any mutations (DDC / + ; + / +), flies expressing the wild-type Vps35 (DDC / + ; wtVPS35 / +), flies expressing the Vps35 (D620N) mutation (DDC / + ; D620N / +), flies expressing the LRRK2 (I2020T) mutation (DDC / +; + / LRRK2), flies expressing both the Vps35 (D620N) mutation and the LRRK2 (I2020T) mutation (DDC / + ; D620N / LRRK2), and flies expressing the wild-type Vps35 and the LRRK2 (I2020T) mutation (DDC / + ; wtVPS35 / LRRK2) to examine the effects of only the Vps35 mutation, then only the LRRK2 mutation, and then the interplay of the Vps35 wild-type and mutant forms with the mutant LRRK2 on locomotor functions in *Drosophila*. Climbing ability was categorized as normal, impaired, or rescued.

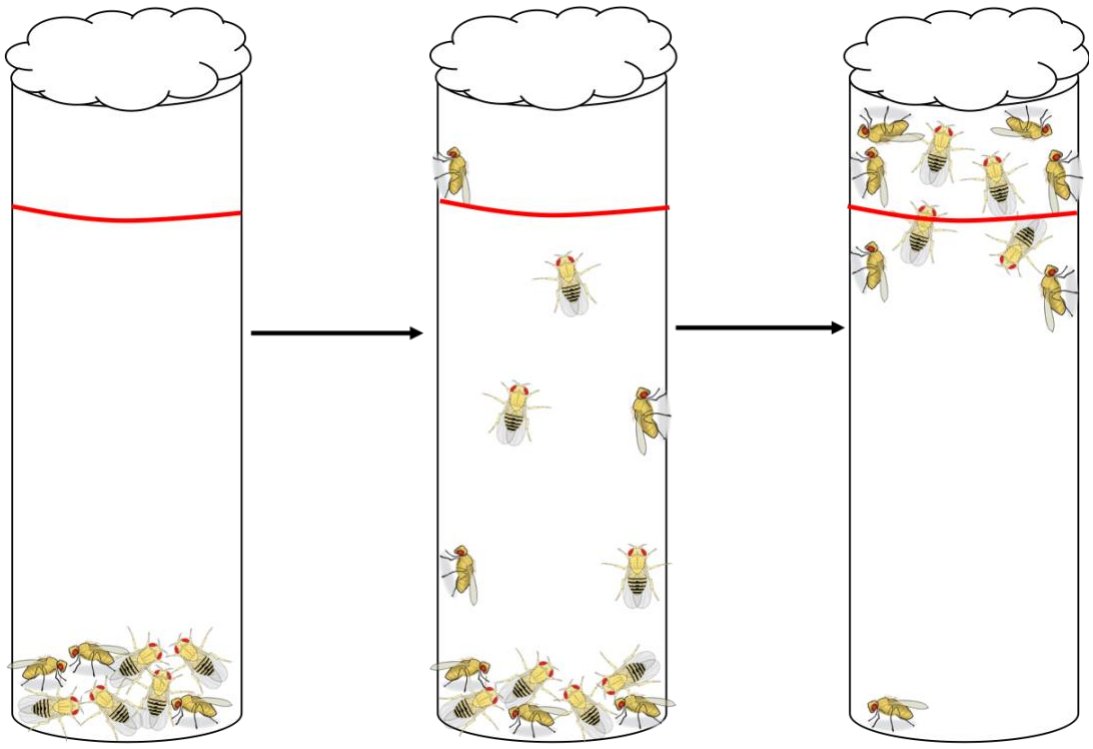

Figure 4. A representation of the climbing assay experimental setup. The vial, with 20 flies, is tapped three times to bring the flies to the bottom of the vial. As they

begin to climb up the vial, the number of flies that cross the red line in 10s and 20s are recorded and filmed.

b) Eye Pigmentation Analysis

An eye pigmentation analysis is used to assess the phenotypic effects of neurodegeneration. As previously stated, the photoreceptor neurons of the *Drosophila* compound eye manifest neurodegeneration through pigmentation loss, disorganization of bristles and/or ommatidia components (also called the "rough eye" phenotype), and the presence of black lesions.

The GMR-Gal4 system was used to drive the expression of transgenes of interest in the *Drosophila* eye. The eye pigmentation analysis was assessed in wild-type flies without any mutations (GMR / + ; + / +), flies expressing the wild-type Vps35 (GMR / + ; wtVPS35 / +), flies expressing the Vps35 (D620N) mutation (GMR / + ; D620N / +), flies expressing the LRRK2 (I2020T) mutation (GMR / +; + / LRRK2), flies expressing both the Vps35 (D620N) mutation and the LRRK2 (I2020T) mutation (GMR / + ; D620N / LRRK2), and flies expressing the wild-type Vps35 and the LRRK2 (I2020T) mutation (GMR / + ; wtVPS35 / LRRK2). Pigmentation loss was categorized as none, low, high, or rescued. A rough eye phenotype was noted as glossy or misshapen eyes. The presence of black lesions was also noted.

IV. COMPOSITE EXPERIMENTAL FINDINGS

a) Proposed mechanism of mutant Vps35 (D620N)-induced neurodegeneration

The retromer complex is necessary for proper recruitment of the WASH complex to the endosomal membrane through the binding of Vps35 to Fam21. Evidence has shown, however, that the expression of the Vps35 (D620N) mutation impairs retromer association with the WASH complex, due to a large decrease in affinity of mutated Vps35 for Fam21 of the WASH complex (McGough et al., 2014, Zavodszky et al., 2014).

By examining the effect of the Vps35 (D620N) mutation on both the endosome-to-TGN transport of CIMPR and the endosome-to-plasma membrane transport of GLUT1, McGough et al. found that the mutated Vps35 impaired endosome-to-TGN transport but did not affect the endosome-to-plasma membrane transport (2014). Further experiments established that the Vps35 (D620N) does not affect the formation of the cargo-recognition complex of the retromer–in that it demonstrates an ability to bind to Vps26 and Vps29 with the same affinity and thermodynamic properties as wild-type Vps35–nor does it inhibit the endosomal localization of Vps35 (Fig 5) (Zavodzsky et al.,2014; Follett et al., 2016).

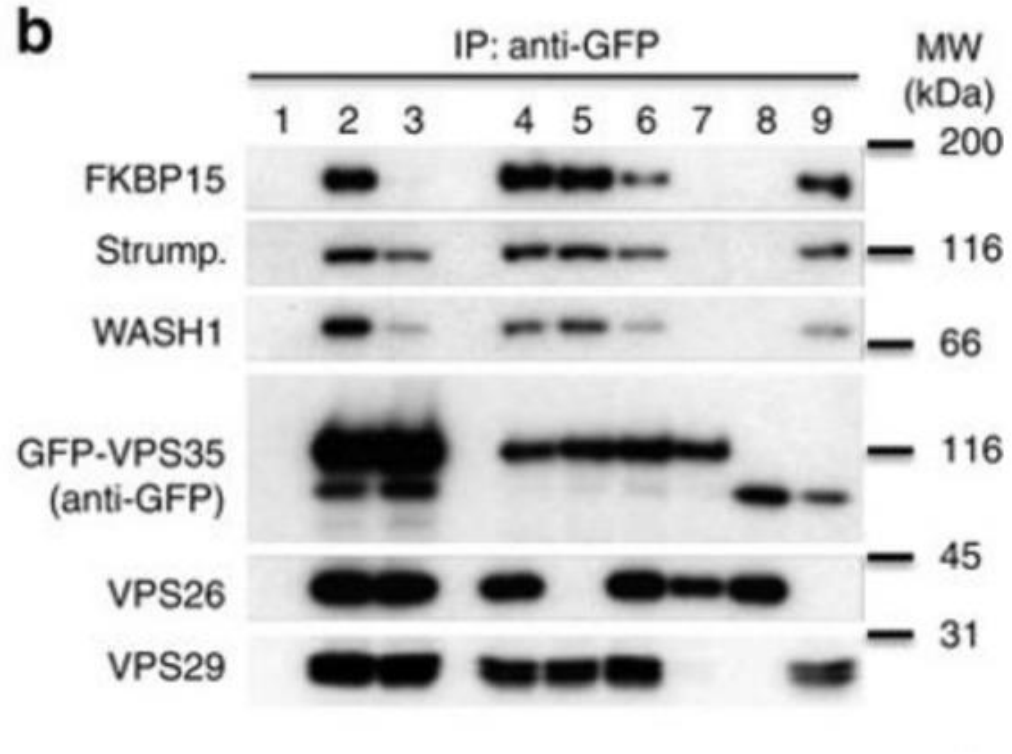

Figure 5. The effect of mutant Vps35 (D620N) on association with proteins of the CRC and WASH complex. Lanes 2 and 4 represent stably transfected and transiently transfected fluorescently tagged wild type VPS35, respectively. Wild type Vps35 shows normal association with the CRC proteins Vps26 and Vps29, as well as the WASH proteins Strump (strumpellin) and Wash1. Lanes 3 and 6 represent stably transfected and transiently transfected fluorescently tagged mutant Vps35 (D620N), respectively. The D620N mutant shows normal association with the CRC proteins Vps26 and Vps29 but shows decreased association with the WASH proteins strumpellin and Wash1 (Figure courtesy of Zavodszky et al., 2014).

The impaired association of the retromer with the WASH complex, however, disrupts autophagy through abnormal Atg9 localization and trafficking. Atg9 normally colocalizes with both Vps35 on sorting endosomes–operating through some of the same domains at which the retromer functions–and with the WASH complex on endosomes. Impaired WASH recruitment to the endosomal membrane, as a result of the Vps35 D620N mutation, thereby perturbs proper trafficking of Atg9

(Zavodszky et al., 2014). Mutant D620N cells show significantly increased abnormal colocalization between Atg9 and TGN46, a marker for the TGN (Fig 6).

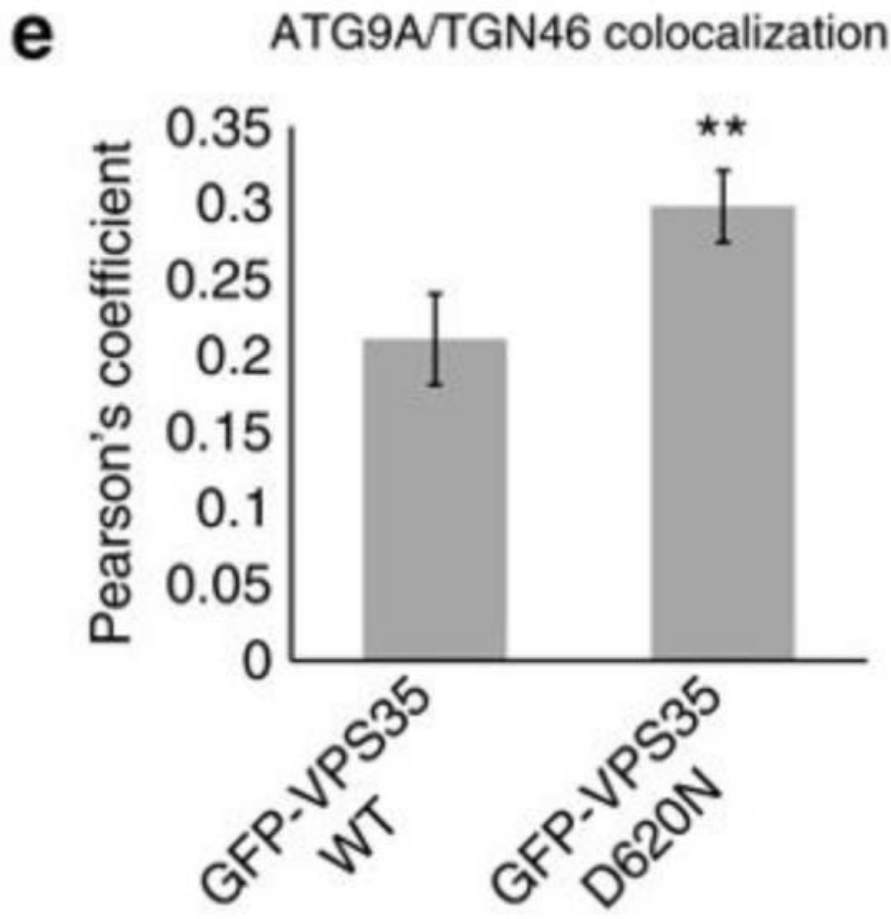

Figure 6. The Vps35 (D620N) mutation impairs proper trafficking and localization of ATG9A. Under normal conditions, ATG9A localizes to the TGN. In cells expressing wild-type Vps35, ATG9A showed normal colocalization with the TGN marker TGN46. In cells expressing mutant Vps35 (D620N), ATG9A showed significantly increased colocalization with the TGN (Figure courtesy of Zavodszky et al., 2014).

This abnormally localization of Atg to the TGN suggests that Atg9 could be trapped in a perinuclear compartment. Impaired trafficking of Atg disrupts proper

autophagosome formation–a deficit similarly observed with other PD-linked mutations (Winslow et al., 2010). In cell lines with mutant Vps35, there is a consistently higher percentage of transfected cells with aggregates, demonstrating a defect in autophagosome formation and autophagic clearance. The impaired association of retromer with the WASH complex which causes abnormal autophagy and a subsequent build-up of toxic protein aggregates and other cellular components as a result of the Vps35 (D620N) mutation seems a plausible mechanism by which mutant Vps35 induces neurodegeneration (Fig 7).

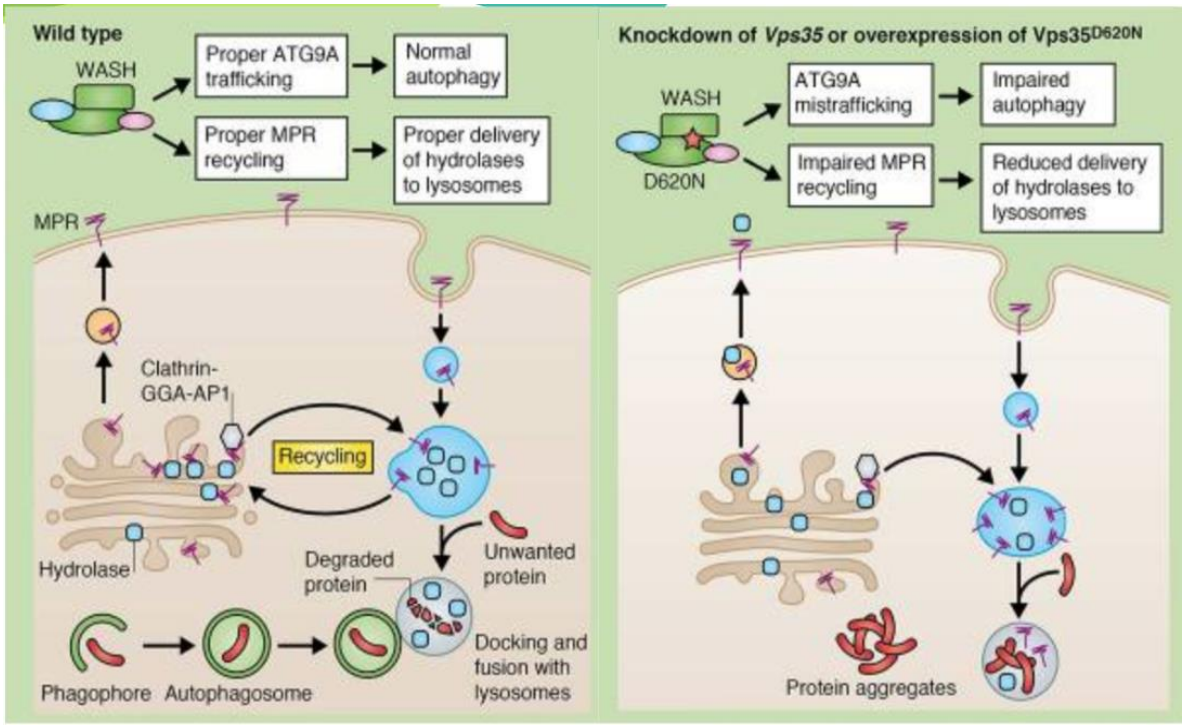

Figure 7. A comparative schematic of the downstream effects of wild-type Vps35 and Vps35 (D620N) mutation. On the left, wild-type Vps35 allows for correct association of the retromer with the WASH complex, leading to proper Atg9 trafficking and normal autophagy. With normal autophagy, autophagosomes fuse with lysosomes to degrade unwanted proteins. On the right, the Vps35 (D620N) mutation disrupts association of the retromer with the WASH complex, leading to

improper Atg9 trafficking and impaired autophagy. This impaired autophagy condition is characterized by impaired autophagosome formation causing a build-up of protein aggregates (Figure courtesy of Wang & Bellen, 2015).

b) LRRK2-linked neurodegeneration and association with Vps35

LRRK2 mutations recapitulate the fundamental pathological characteristics of neurodegenerative diseases. Ectopic expression of LRRK2 causes substantial loss of dopaminergic neurons as well as locomotor defects in *Drosophila* mutants.

To gain a better understanding of the physiological and pathological functions of LRRK2 mutations, many groups study transgenic *Drosophila* models. Utilizing the *Drosophila* UAS/Gal4 system of targeted transgene expression and selective expression of genes allows for generation of transgenic *Drosophila* models overexpressing the human LRRK2 (hLRRK2) with a specific kinase domain mutation of isoleucine to threonine at residue 2020 (I2020T).

Through examination of four posterior paired dopaminergic clusters (dorsolateral posterior protocerebral (PPL1), lateral posterior porotocerebral (PPL2) and two dorsomedial posterior protocerebral clusters (PPM1/2 and PPM3), Venderova et al. found that expression of LRRK2 mutations, under the control of the tyrosine hydroxylase (TH) gene promotor, causes a loss of dopaminergic neurons (Fig 7) (2009).

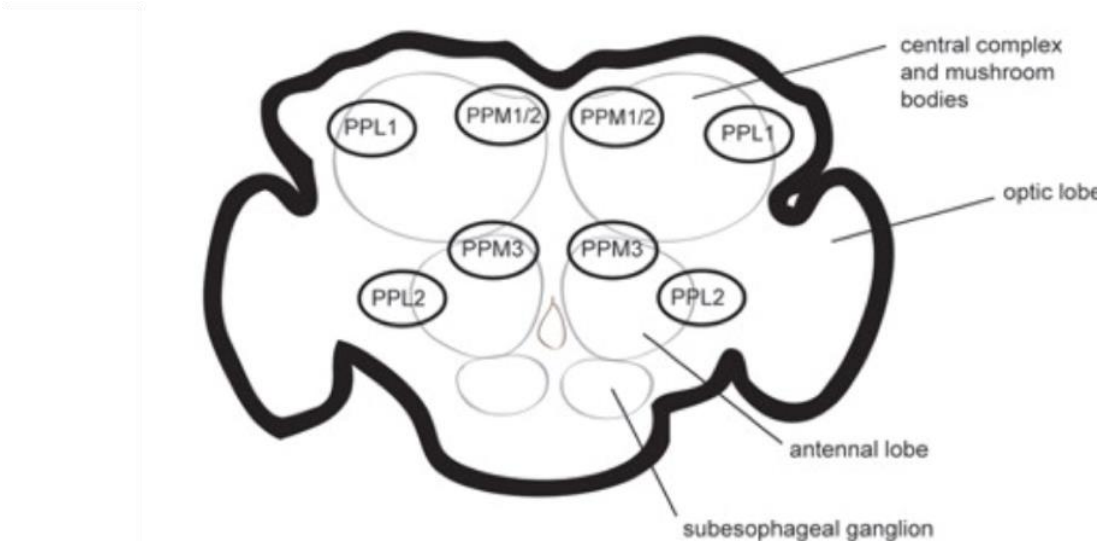

Figure 8. Schematic representation of the fly brain, highlighting the four posterior paired dopaminergic clusters: PPL1, PPL2, PPM1/2, and PPM3 (Figure courtesy of Venderova et al., 2009).

While the dopaminergic clusters of control fly brains did not show any significant changes in number of morphology of neurons during aging, analysis of mutant fly brains revealed loss of dopaminergic neurons most noticeably in the PPM1/2 cluster and the PPL1 cluster, with the most prominent loss of neurons seen in flies expressing the LRRK2 (I2020T) mutation (Fig 9).

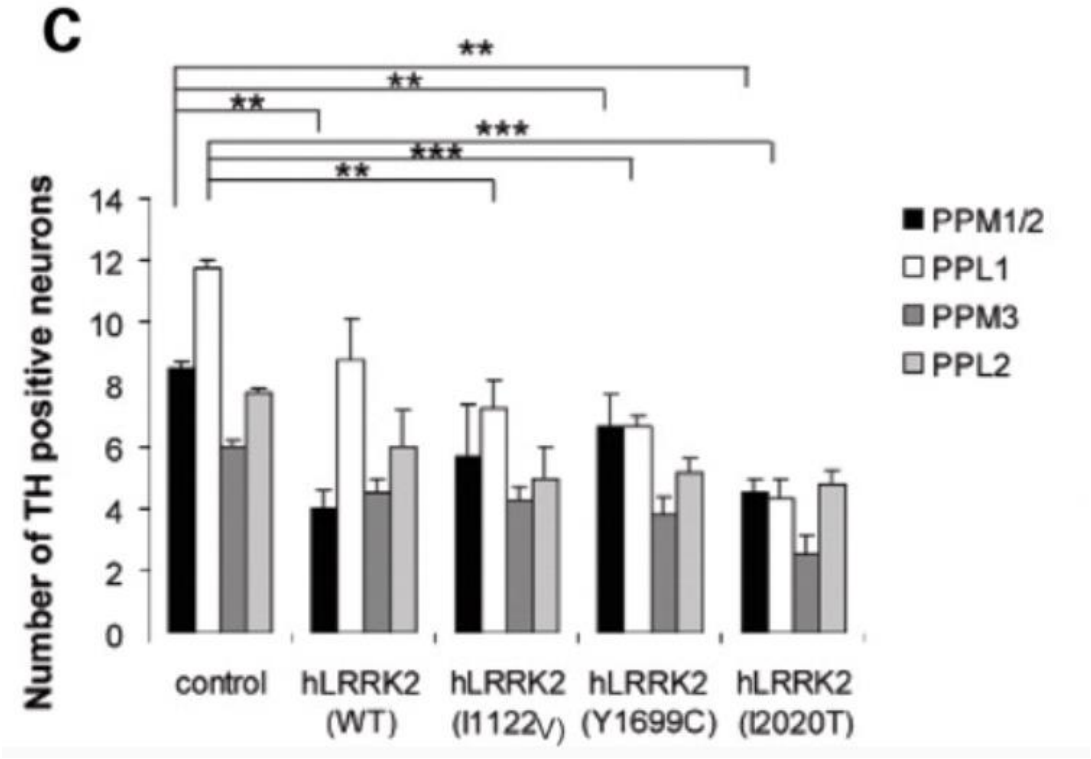

Figure 9. Mutant LRRK2 expression induces dopaminergic neuron death. The number of TH positive dopaminergic neurons was significantly lower after 10 days in the two dorsomedial posterior protocerebral clusters (PPM1/2) and the dorsolateral posterior protocerebral cluster (PPL1) of transgenic flies expressing the LRRK2 12020T mutation (Figure courtesy of Venderova et al., 2009).

LRRK2 mutations were also found to impair *Drosophila* locomotor activity. Using the climbing assay technique, locomotor defects were quantified through the number of flies that crossed the horizontal line on the vial within a certain amount of time. While the effects of the mutant LRRK2 were complex as a result of the age of the fly, climbing ability in all mutant lines was impaired, with the greatest locomotor deficit seen in LRRK2 (I2020T) mutants (Fig 10) (Venderova et al., 2009).

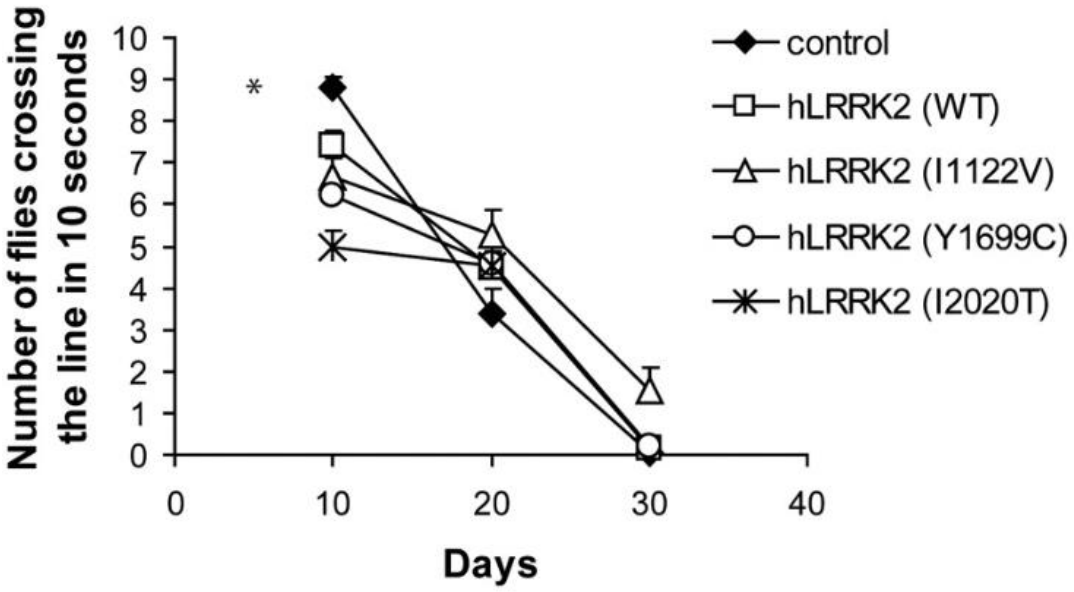

Figure 10. Mutant LRRK2 expression causes locomotor defects. A climbing assay was used to analyze the effects of the LRRK2 mutations on locomotor activity. Flies with the mutant LRRK2 I2020T exhibited the greatest locomotor deficit, quantified through the number of flies that crossed the horizontal line in 10 seconds in a climbing assay (Figure courtesy of Venderova et al., 2009).

While younger transgenic flies were able to climb as well as non-mutant control flies, their performance deteriorated at a much faster rate than the control flies (Liu et al., 2008).

LRRK2 mutations were further shown to cause retinal degeneration, as loss of pigmentation, disorganization of the eye structure, and black lesions in *Drosophila* eyes phenotypically manifested the neurodegenerative effects of the mutations. Ectopic expression of the LRRK2 (I2020T) mutation in the fly eye caused loss of pigmentation and, in some cases led to the development of black lesions. Further, sectional examination of the ommatidial structure demonstrated disruption of the well-structured photoreceptor arrangement in flies expressing the LRRK2 (I2020T) mutation. With glossy and rough eye surfaces in addition to disorganization of the

bristles surrounding each photoreceptor neuron, flies with the LRRK2 (I2020T) mutation ultimately displayed sever neurodegenerative effects (Fig 11) (Venderova et al., 2009).

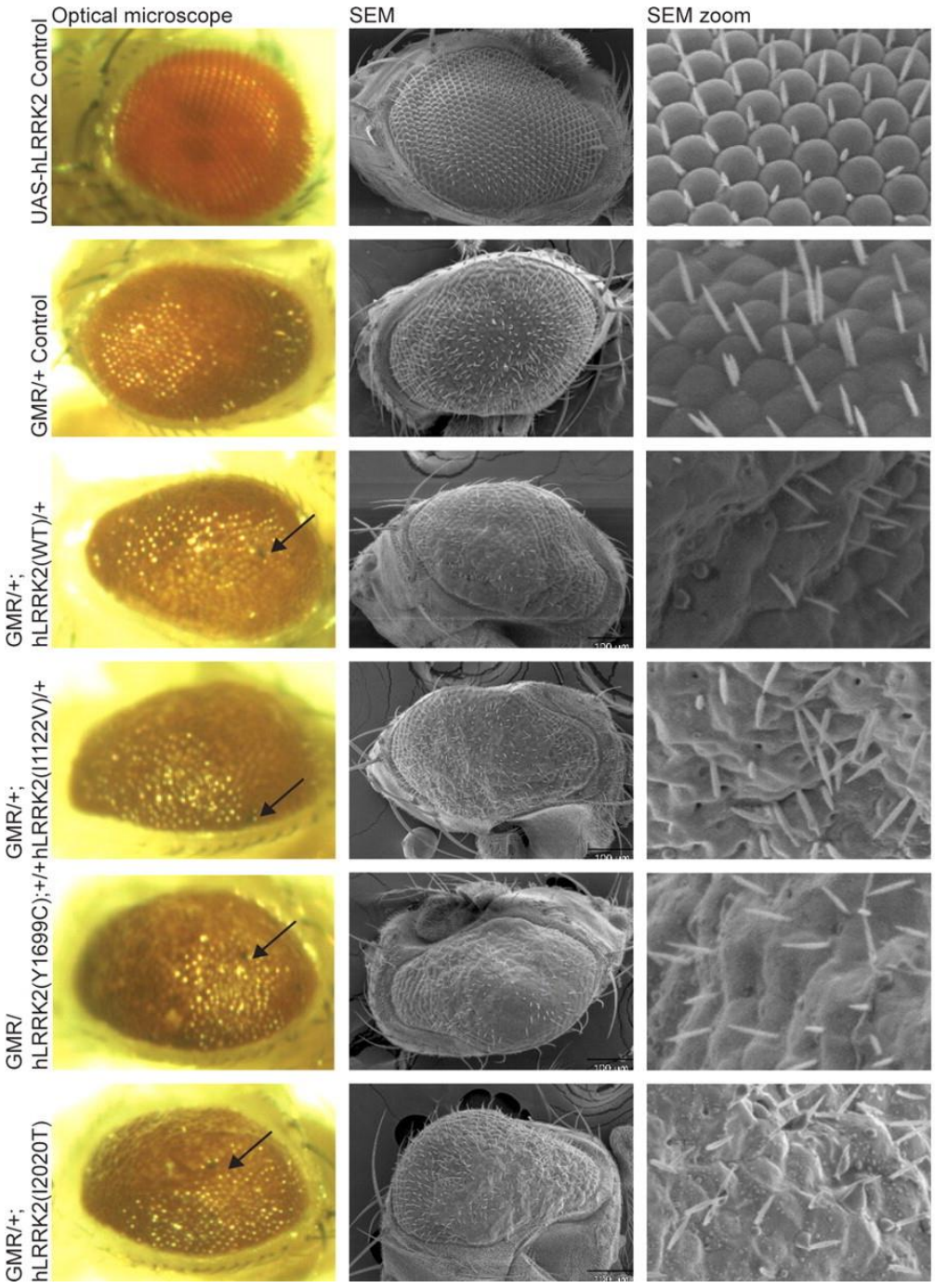

Figure 11. Mutant LRRK2 expression causes pigmentation loss and structural abnormalities in the *Drosophila* eye. Wild-type *Drosophila* eyes exhibit a red

pigmentation and display a highly organized structure of mechanosensory interommatidial bristles. Transgenic *Drosophila* eyes exhibit pigmentation loss as well as a disorganization of bristles (Figure courtesy of Venderova et al., 2009).

Specifically, with experimental evidence to suggest that association of wild-Vps35 is two-fold in their involvement with the retromer and endosomal trafficking and sorting as well as their causal links to PD pathology, a focus on the interactions of Vps35 and LRRK2 and the downstream effect could elucidate the molecular mechanisms of neurodegenerative disease pathogenesis.

c) Wild-type Vps35 rescues neurodegenerative effects of PD-linked LRRK2 mutation

Recent evidence has shown that expression of wild-type Vps35 rescues the PD-associated characteristics of flies expressing mutant LRRK2, demonstrating that Vps35 and LRRK2 operate within common cellular pathways and genetically interact to produce pathological features of PD.

As previously shown, the expression of the LRRK2 (I2020T) mutation causes neurodegeneration of photoreceptor neurons in the *Drosophila* eye, manifest through pigmentation loss, structural defects, and the appearance of black lesions. However, overexpression of Vps35 completely rescued the eye phenotype of LRRK2 mutant flies. When Vps35 was expressed, none of the flies with the mutant LRRK2 exhibited black lesions. Expressing Vps35 also ameliorated some of the pigmentation loss seen in flies with the LRRK2 (12020T) mutation, as there was less yellow coloration compared to flies only expressing the LRRK2 mutation (Fig 12) (Linhart et al., 2014).

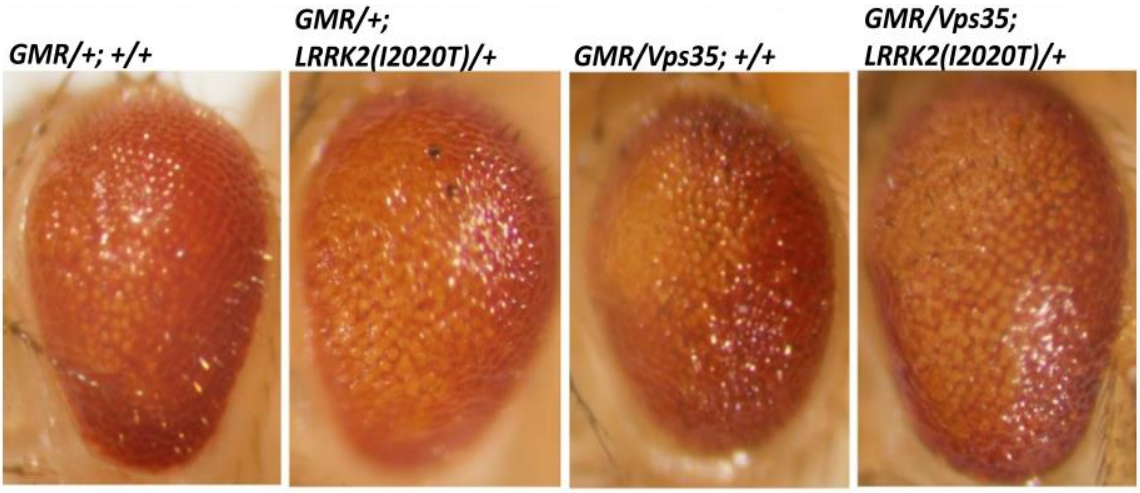

Figure 12. Eye-specific overexpression of wild-type Vps35 rescues the black lesion phenotype caused by the LRRK2 (I2020T) mutation. The use of the GMR promoter allowed for targeted expression of transgenes in the eye. The *Drosophila* eye displays black lesions when only the mutant LRRK2 is expressed. When wild-type Vps35 is expressed with the mutant LRRK2, there are no black lesions (Figure courtesy of Linhart et al., 2014).

Expression of the LRRK2 (I2020T) mutation also causes locomotor defects. Transgenic flies with the LRRK2 mutation expressed in dopaminergic neurons had significant locomotor defects–manifest through their impaired climbing ability. However, overexpression of Vps35 rescued the locomotor defects caused by the LRRK2 (I2020T) mutation, as a larger percent of flies expressing wild-type Vps35 and mutant LRRK2 were able to cross the line in 5 seconds as compared to transgenic flies not expressing the wild-type Vps35 (Fig 13) (Linhart et al., 2014).

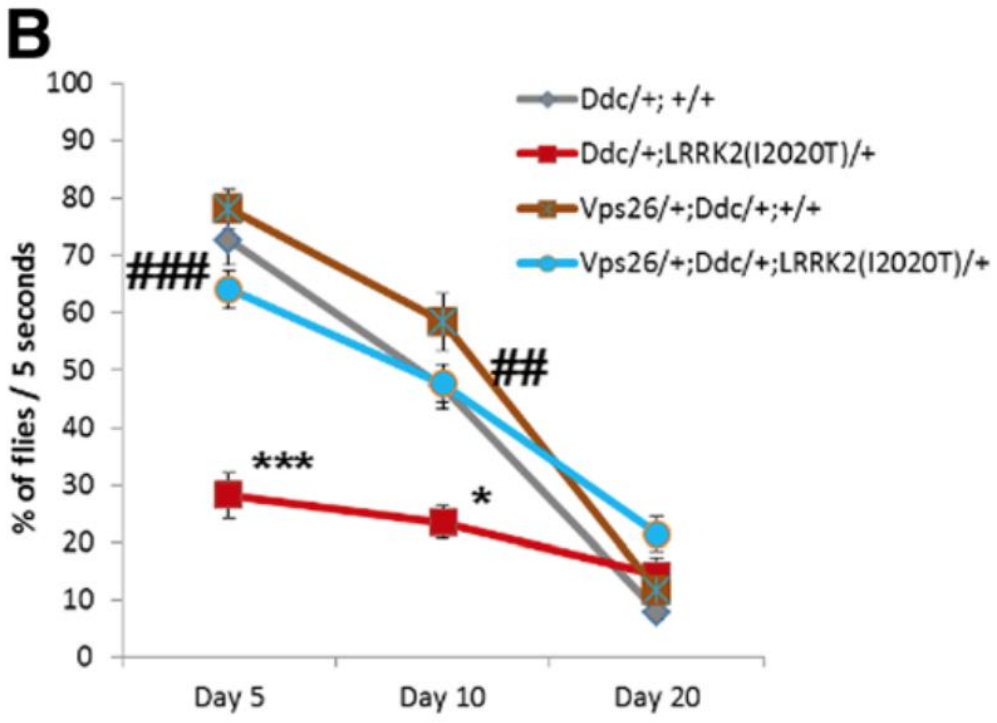

Figure 13. Overexpression of wild-type Vps35 rescues the locomotor defects caused by mutant LRRK2 I2020T. The use of the DDC driver allowed for targeted

expression of the transgenes in dopaminergic neurons. Flies expressing the LRRK2 mutation had a statistically significant lower percentage of flies cross the line in 5 seconds compared to wild-type control flies in a climbing assay. Flies expressing the wild-type Vps35 and LRRK2 mutation had a statistically significant increase in percentage of flies that crossed the line in 5 seconds compared to flies only expression the LRRK2 mutation (Figure courtesy of Linhart et al., 2014).

V. IMPLICATIONS AND FUTURE DIRECTIONS

While the molecular mechanisms and cellular pathways underlying Parkinson's disease pathology are still not fully understood, evidence of a functional interaction between Vps35 and LRRK2 provides immense insight into the ways PD-linked genes may interplay within a common pathway and also strongly implicates the retromer complex in the progression of neurodegenerative diseases, especially PD.

Continued research in the field of Parkinson's disease is crucial given the disparity between effective treatments for neurodegenerative disorders and the increasing prevalence of these diseases as a greater percentage of the population ages. Emerging evidence pointing to the integral role of the retromer in PD pathology has allowed for a greater focus on its cellular pathways and molecular mechanisms. While the specific Vps35 (D620N) mutation has been implicated in PD pathology as a result of its disruption of WASH and retromer association and impairment of proper autophagy, both the retromer and WASH complexes have many other components that also play important roles in endosomal sorting and recycling.

Preliminary data has shown links between PD-like pathology and mutations in various components of the retromer and WASH complexes, including the receptor-mediated endocytosis-8 (RME-8), the family with sequence similarity 21 (FAM21), and the WAS protein family homolog 1 (WASH1). Broader research into the role these genes play in the retromer and WASH complexes in addition to more focused research into the ways their variants associate with PD progression could allow for a better insight into the molecular mechanisms of PD pathogenesis.

Further research into the functional relationship between Vps35 and LRRK2 could also promise exciting findings. A more robust understanding of the ways in which overexpression of wild-type Vps35 ameliorates PD pathologies, as well as research into the plausible ability of other genes to rescue detrimental symptoms, could point toward certain genes as potential targets for therapeutic treatments of PD.

WORKS CITED

Alegre-Abarrategui, Javier, et al. “LRRK2 Regulates Autophagic Activity and Localizes to Specific Membrane Microdomains in a Novel Human Genomic Reporter Cellular Model.” *Human Molecular Genetics*, vol. 18, no. 21, 1 Nov. 2009, pp. 4022–4034., doi:10.1093/hmg/ddp346.

Ascherio, Alberto, and Michael Schwarzschild. “The Epidemiology of Parkinson's Disease: Risk Factors and Prevention.” *The Lancet Neurology*, vol. 15, no. 12, Nov. 2016, pp. 1257–1272.

Brazier, Yvette. “Parkinson's Disease: Early Signs, Causes, and Risk Factors.” Edited by Nancy Carteron, *Medical News Today*, MediLexicon International, 29 Oct. 2018, www.medicalnewstoday.com/articles/323396.php.

Cauchi, Ruben J., and Marcel Van Den Heuvel. “The Fly as a Model for Neurodegenerative Diseases: Is It Worth the Jump?” *Neurodegenerative Diseases*, vol. 3, no. 6, Oct. 2006, pp. 338–356., doi:10.1159/000097303.

Collins, Brett M. “The Structure and Function of the Retromer Protein Complex.” *Traffic*, vol. 9, no. 11, Nov. 2008, pp. 1811–1822., doi:10.1111/j.1600-0854.2008.00777.x.

Follett, Jordan, et al. “Parkinson Disease-Linked Vps35 R524W Mutation Impairs the Endosomal Association of Retromer and Induces α-Synuclein Aggregation.” *Journal of Biological Chemistry*, vol. 291, no. 35, 26 Aug. 2016, pp. 18283–18298., doi:10.1074/jbc.m115.703157.

Follett, Jordan, et al. “The Vps35 D620N Mutation Linked to Parkinson's Disease Disrupts the Cargo Sorting Function of Retromer.” *Traffic*, vol. 15, no. 2, 23 Oct. 2013, pp. 230–244., doi:10.1111/tra.12136.

Hansen, Malene, et al. “Autophagy as a Promoter of Longevity: Insights from Model Organisms.” *Nature Reviews Molecular Cell Biology*, vol. 19, no. 9, 13 July 2018, pp. 579–593., doi:10.1038/s41580-018-0033-y.

Harbour, Michael E., and Matthew N. J. Seaman. “Evolutionary Variations of VPS29, and Their Implications for the Heteropentameric Model of Retromer.” *Communicative & Integrative Biology*, vol. 4, no. 5, 1 Sept. 2011, pp. 619–622., doi:10.4161/cib.16855.

Hirth, Frank. “Drosophila Melanogaster in the Study of Human Neurodegeneration.” *CNS & Neurological Disorders - Drug Targets*, vol. 9, no. 4, Aug. 2010, pp. 504–523., doi:10.2174/187152710791556104.

Imai, Yuzuru, et al. “Animal Models of Parkinson's Disease.” *Parkinson's Disease*, vol. 2011, 14 Nov. 2011, pp. 1–2., doi:10.4061/2011/364328.

Li, Jie-Qiong, et al. “The Role of the LRRK2 Gene in Parkinsonism.” *Molecular Neurodegeneration*, vol. 9, no. 47, 12 Nov. 2014, p. 47., doi:10.1186/1750-1326-9-47.

Linhart, Radek, et al. “Vacuolar Protein Sorting 35 (Vps35) Rescues Locomotor Deficits and Shortened Lifespan in Drosophila Expressing a Parkinson’s Disease Mutant of Leucine-Rich Repeat Kinase 2 (LRRK2).” *Molecular Neurodegeneration*, vol. 9, no. 23, 9 May 2014, pp. 1–10., doi:10.1186/1750-1326-9-23.

Liu, Z., et al. “A Drosophila Model for LRRK2-Linked Parkinsonism.” *Proceedings of the National Academy of Sciences*, vol. 105, no. 7, 7 Feb. 2008, pp. 2693–2698., doi:10.1073/pnas.0708452105.

“LRRK2 Gene.” *Genecards.org*, Weizmann Institute of Science, www.genecards.org/cgi-bin/carddisp.pl?gene=LRRK2.

Lynch-Day, M. A., et al. "The Role of Autophagy in Parkinson's Disease." *Cold Spring Harbor Perspectives in Medicine*, vol. 2, no. 4, Apr. 2012, doi:10.1101/cshperspect.a009357.

Macleod, David A., et al. "RAB7L1 Interacts with LRRK2 to Modify Intraneuronal Protein Sorting and Parkinson's Disease Risk." *Neuron*, vol. 77, no. 3, 6 Feb. 2013, pp. 425–439., doi:10.1016/j.neuron.2012.11.033.

Marcogliese, Paul C., et al. "LRRK2(I2020T) Functional Genetic Interactors That Modify Eye Degeneration and Dopaminergic Cell Loss in Drosophila." *Human Molecular Genetics*, vol. 26, no. 7, 2 Feb. 2017, pp. 1247–1257., doi:10.1093/hmg/ddx030.

Marras, C., et al. "Prevalence of Parkinson's Disease across North America." *Npj Parkinson's Disease*, vol. 4, no. 1, 10 July 2018, doi:10.1038/s41531-018-0058-0.

Maruzs, Tamás, et al. "Retromer Ensures the Degradation of Autophagic Cargo by Maintaining Lysosome Function in Drosophila." *Traffic*, vol. 16, no. 10, 14 July 2015, pp. 1088–1107., doi:10.1111/tra.12309.

Mcgough, Ian J., et al. "Retromer Binding to FAM21 and the WASH Complex Is Perturbed by the Parkinson Disease-Linked VPS35(D620N) Mutation." *Current Biology*, vol. 24, no. 14, 21 July 2014, pp. 1670–1676., doi:10.1016/j.cub.2014.06.024.

Mishra, Monalisa, and Elisabeth Knust. "Analysis of the Drosophila Compound Eye with Light and Electron Microscopy." *Methods in Molecular Biology Retinal Degeneration*, vol. 935, 2013, pp. 161–182., doi:10.1007/978-1-62703-080-9_11.

Mizushima, N. "Autophagy: Process and Function." *Genes & Development*, vol. 21, no. 22, 2007, pp. 2861–2873., doi:10.1101/gad.1599207.

Mizushima, Noboru, and Masaaki Komatsu. “Autophagy: Renovation of Cells and Tissues.” *Cell*, vol. 147, no. 4, 11 Nov. 2011, pp. 728–741., doi:10.1016/j.cell.2011.10.026.

Muñoz-Soriano, Verónica, and Nuria Paricio. “Drosophila Models of Parkinson's Disease: Discovering Relevant Pathways and Novel Therapeutic Strategies.” *Parkinson's Disease*, vol. 2011, 7 Jan. 2011, pp. 1–14., doi:10.4061/2011/520640.

Papinski, Daniel, et al. “Early Steps in Autophagy Depend on Direct Phosphorylation of Atg9 by the Atg1 Kinase.” *Molecular Cell*, vol. 53, no. 3, 6 Feb. 2014, pp. 471–483., doi:10.1016/j.molcel.2013.12.011.

Rahman, Abir A., and Brad E. Morrison. “Contributions of VPS35 Mutations to Parkinson’s Disease.” *Neuroscience*, vol. 401, 1 Mar. 2019, pp. 1–10., doi:10.1016/j.neuroscience.2019.01.006.

Sang, Tzu-Kang, and George R. Jackson. “Drosophila Models of Neurodegenerative Disease.” *NeuroRX*, vol. 2, no. 3, July 2005, pp. 438–446., doi:10.1602/neurorx.2.3.438.

Seaman, M. N. J. “The Retromer Complex - Endosomal Protein Recycling and Beyond.” *Journal of Cell Science*, vol. 125, no. 20, 2012, pp. 4693–4702., doi:10.1242/jcs.103440.

Seaman, Matthew N.j., et al. “Retromer-Mediated Endosomal Protein Sorting: All WASHed up!” *Trends in Cell Biology*, vol. 23, no. 11, 28 May 2013, pp. 522–528., doi:10.1016/j.tcb.2013.04.010.

Swarbrick, James D., et al. “VPS29 Is Not an Active Metallo-Phosphatase but Is a Rigid Scaffold Required for Retromer Interaction with Accessory Proteins.” *PLoS ONE*, vol. 6, no. 5, 24 May 2011, doi:10.1371/journal.pone.0020420.

Vardarajan, Badri N., et al. “Identification of Alzheimer Disease-Associated Variants in Genes That Regulate Retromer Function.” *Neurobiology of Aging*, vol. 33, no. 9, Sept. 2012, doi:10.1016/j.neurobiolaging.2012.04.020.

Venderova, K., et al. “Leucine-Rich Repeat Kinase 2 Interacts with Parkin, DJ-1 and PINK-1 in a Drosophila Melanogaster Model of Parkinson's Disease.” *Human Molecular Genetics*, vol. 18, no. 22, 19 Aug. 2009, pp. 4390–4404., doi:10.1093/hmg/ddp394.

Vilariño-Güell, Carles, et al. “VPS35 Mutations in Parkinson Disease.” *The American Journal of Human Genetics*, vol. 89, no. 1, 15 July 2011, pp. 162–167., doi:10.1016/j.ajhg.2011.06.001.

Wang, Fei, et al. “WASH Complex Regulates Arp2/3 Complex for Actin-Based Polar Body Extrusion in Mouse Oocytes.” *Scientific Reports*, vol. 4, no. 1, 7 July 2014, pp. 1–8., doi:10.1038/srep05596.

Wang, S., and H. J. Bellen. “The Retromer Complex in Development and Disease.” *Development*, vol. 142, no. 14, 2015, pp. 2392–2396., doi:10.1242/dev.123737.

Williams, Erin T., et al. “VPS35, The Retromer Complex and Parkinson’s Disease.” *Journal of Parkinson's Disease*, vol. 7, no. 2, 13 Jan. 2017, pp. 219–233., doi:10.3233/jpd-161020.

Winslow, Ashley R., et al. “α-Synuclein Impairs Macroautophagy: Implications for Parkinson’s Disease.” *The Journal of Cell Biology*, vol. 190, no. 6, 20 Sept. 2010, pp. 1023–1037., doi:10.1083/jcb.201003122.

Wund, Matthew, and Sean Zera. “Critter Catalog.” *BioKIDS - Kids' Inquiry of Diverse Species, Glaucomys Sabrinus, Northern Flying Squirrel: INFORMATION*, 2004, www.biokids.umich.edu/critters/Condylura_cristata/.

Xiong, Yulan, and Jianzhong Yu. "Modeling Parkinson's Disease in Drosophila: What Have We Learned for Dominant Traits?" *Frontiers in Neurology*, vol. 9, no. 228, 9 Apr. 2018, doi:10.3389/fneur.2018.00228.

Zavodszky, Eszter, et al. "Mutation in VPS35 Associated with Parkinson's Disease Impairs WASH Complex Association and Inhibits Autophagy." *Nature Communications*, vol. 5, no. 1, 13 May 2014, doi:10.1038/ncomms4828.