Characterization of *CG34126:* the *Drosophila melanogaster* homologue of a novel Parkinson Disease gene

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Abstract

Parkinson Disease (PD) is a progressive and debilitating neurodegenerative disease characterized by the inadequate function or the loss of dopamine-producing neurons in the human midbrain. PD has a complex etiology including aberrant protein inclusion, mitochondrial dysfunction, oxidative stress, and defects in protein trafficking, which may occur due to genetic or non-genetic factors. Recently identified PD causative genes can be evaluated in model organisms, through which pathophysiological interactions with previously discovered PD genes may be determined. A new gene associated with PD, *UHRF1BP1L*, has a putative role in mitochondrial dynamics and protein homeostasis, similar to several other well-established PD genes. I investigated *CG34126,* the only homologue of *UHRF1BP1L* in *Drosophila melanogaster*, in an attempt to model PD using the *UAS-GAL4* system of inducible gene expression in the dopaminergic neurons as well as during the development of the eye. In brief, RNAi-mediated knockdown of *CG34126* can reduce lifespan and locomotor ability over time, and the overexpression of *CG34126* directed by the *Dopa decarboxylase (Ddc)-Gal4* transgene leads to reduced longevity and climbing ability throughout life. However, overexpression of *CG34126* partially rescues the *parkin*-RNA-interference model of PD by increasing both longevity and locomotor ability over time. No neurodevelopmental defect was observed through a detailed biometric analysis of eye phenotypes, either from overexpression or knockdown of *CG34126*. As the altered ectopic expression of *CG34126* can either positively or negatively influence ageing and locomotion, the consequences of altering *UHRF1BP1L* function in the fly contributes a better understanding of PD and general ageing mechanisms.

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Table of Contents

Abstract ii

Acknowledgements iii

Table of Contents iv

List of Figures vii

List of Tables vii

List of Abbreviations and Symbols ix

Introduction 1

Parkinson Disease 1

Causative and associated genes of PD 2

Drosophila melanogaster as a model organism for neurodegenerative diseases 3

Modelling Parkinson Disease in *Drosophila melanogaster* 6

Models of PD 9

The α-synuclein model 9

The *parkin* model 11

The *PINK1* model 13

The *DJ-1* model 14

The *LRRK2* model 16

The role of the mitochondria in PD 17

The ubiquitin-proteasome system (UPS) in PD 20

The endoplasmic reticulum and Golgi body link to PD 21

The endosomal-lysosomal regulation in PD 22

Gene of interest 24

Protein trafficking role of *UHRF1BP1L* 27
Mitochondrial dysfunction and *UHRF1BP1L* 28
CG34126 gene in *Drosophila melanogaster* 30
Homeostasis and Parkinson Disease 30
Goals and Objectives 31
Methods and Materials 33
Bioinformatic assessment 33
Drosophila stocks and derivative lines 33
Media and culture 36
Biometric analysis of *Drosophila* eye 36
Lifespan analysis 37
Climbing analysis 37
Results 39
Bioinformatics analysis 39
Identification of the *Drosophila melanogaster* homologue of *UHRF1BP1L* 39
UHRF1BP1L and CG34126 are conserved between vertebrates and invertebrates 46
Analysis of the consequences of altered expression in the developing eye 55
Effects of overexpression and RNA interference of *CG34126* in eye phenotypes 55
Overexpression and inhibition of *CG34126* gene in selected neurons 59
Lifespan analysis 59
The effects of loss-of-function and gain-of-function of *CG34126* 59
Climbing analysis of the control and experimental lines 64
The effects of loss-of-function and gain-of-function of *CG34126* 64
Expression of *CG34126* in the *park* interference model of PD 68

Longevity analysis of the control and experimental lines in the *park* model 68

Overexpression of *CG34126* in dopaminergic neurons rescues *UAS-park-RNAi* mediated loss of longevity 68

Climbing analysis of the control and experimental lines in the *UAS-park-RNAi* model. 70

CG34126 inhibition exacerbates climbing dysfunction in the *UAS-park-RNAi* model 70

CG34126 overexpression rescues climbing ability in *UAS-park-RNAi* model............ 70

Discussion 73

Conclusion 81

References 82

List of Figures

Figure 1. The GAL4-UAS directed expression system. 8

Figure 2. Alignment of protein encoded by *Drosophila melanogaster* CG34126 with the two human UHRF1BP1 and UHRF1BP1L protein. 43

Figure 3. Pairwise Sequence Alignment of UHRF1BP1L and CG34126 domains.......... 45

Figure 4. (A) *Drosophila melanogaster* CG34126 protein (1475 amino acid residues) and (B) *Homo Sapiens* UHRF1BP1L protein (1464 amino acid residues) with conserved domains. 46

Figure 5. Alignment of human UHRF1BP1L protein with similar protein from selected vertebrates and invertebrates.. 54

Figure 6. Eye of critical class of *Drosophila melanogaster* with altered *CG34126* expression visualised by scanning electron microscopy... 56

Figure 7. Biometric analysis of *Drosophila melanogaster* eye with altered *CG34126* expression. 57

Figure 8. Longevity curves of flies with altered expression of *CG34126* in the motor neurons. 61

Figure 9. Longevity curves of flies with altered expression of *CG34126* in the dopaminergic neurons. 62

Figure 10. Longevity curves of flies with altered expression of *CG34126* in the motor neurons. 63

Figure 11. Climbing ability over time of flies with altered expression of *CG34126* in motor neurons. 65

Figure 12. Climbing ability over time of flies with altered expression of *CG34126* in motor neurons. 66

Figure 13. Climbing ability over time of flies with altered expression of *CG34126* in motor neurons. 67

Figure 14. Longevity of flies with inhibition of *park* along with altered expression of *CG34126* in *Ddc-GAL4* expressing neurons... 69

Figure 15. Climbing ability over time of flies with inhibition of *park* along with altered expression of *CG34126* in *Ddc-GAL4* expressing neurons. 71

List of Tables

Table 1: List of established genes responsible for familial forms of Parkinson Disease ... 4

Table 2: Genotypes of all stocks used to characterize *CG34126* in this study 35

Table 3: Biometric analysis of the directed overexpression and RNA interference of *CG34126* in the developing Drosophila eye. 58

Table 4: Comparison of survival of flies with altered expression of *CG34126* by *D42-GAL4* by (Mantel-Cox) Log-rank test. 61

Table 5: Comparison of survival of flies with altered expression of *CG34126* by *Ddc-GAL4* by (Mantel-Cox) Log-rank test. 62

Table 6: Comparison of survival of flies with altered expression of *CG34126* by *TH-GAL4* by (Mantel-Cox) Log-rank test. 63

Table 7: Comparison of climbing ability of flies with altered expression of *CG34126* by *D42-GAL4*. 65

Table 8: Comparison of climbing ability of flies with altered expression of *CG34126* by *Ddc-GAL4*. 66

Table 9: Comparison of climbing ability of flies with altered expression of *CG34126* by *TH-GAL4*. 67

Table 10: Comparison of survival of flies with inhibition of *park* along with altered expression of *CG34126* in *Ddc-GAL4* expressing neurons by (Mental-Cox) Log-rank test. 69

Table 11: Comparison of climbing ability of flies with inhibition of *park* along with altered expression of *CG34126* in *Ddc-GAL4* expressing neurons by (Mental-Cox) Log-rank test. 71

Table 12: Summary of longevity and climbing over time results 72

List of Abbreviations and Symbols

ADP - Adenosine diphosphate

ADPD - Autosomal dominant Parkinson Disease

ARJP - Autosomal recessive juvenile Parkinsonian

ATP - Adenosine triphosphate

BLAST - Basic local alignment search tool

CDD - Conserved domain database

CI - Confidence interval

C-terminal - Carboxy- terminal

CoQ - Co-enzyme Q

CNS - Central nervous system

DA - Dopaminergic

Ddc - DOPA decarboxylase

DNA - Deoxyribonucleic acid

EIF4G1 - Eukaryotic translation initiation factor 4 gamma 1

EOAR - Early-onset autosomal recessive

EOPD - Early onset Parkinson Disease

FNIII - Fibronectin type III-like fold

FPD - Familiar form of Parkinson Disease

GAL4 - Galactose-inducible genes

GMR - Glass multiple reporter

GOF - Gain-of-function

Hsp70 - Heat shock protein 70

HTRA2 - High temperature requirement A serine peptidase 2

HGNC - HUGO gene nomenclature committee

LacZ - Lactose inducible gene

LB - Lewy body

LN - Lewy neurite

LRRK2 - Leucine rich repeat kinase 2

LOF - Loss-of-function

mtDNA - mitochondrial DNA

MAPK - Mitogen-activated protein kinase

NCBI - National center for biotechnology information

NRF-1 - Nuclear respiratory factor-1

N-terminal - Nitrogen terminal

PD - Parkinson Disease

PINK1- PTEN Induced putative kinase 1

PLA2G6 - Phospholipase A2, group 6

RING - Really interesting new gene

RNAi - RNA interference

ROS - Reactive oxygen species

SEM - Standard error of the mean

SNARE - Soluble NSF attachment protein

NLS - Nuclear localization signal

NSF - N-ethylmaleimide sensitive factor

SNpc - Substantia nigra pars compacta

SNCA - Synuclein alpha

tBLASTn - Translated nucleotide basic local alignment search tool

UCHL1 - Ubiquitin carboxyl-terminal hydrolase isozyme L1

UAS - Upstream activating sequence

VPS35 - Vacuolar protein sorting 35

VPS13C - vacuolar protein sorting 13C

YPT1 - Yeast protein two

°C - Degree Celsius

α-Synuclein - Alpha-synuclein

kDa - kilo Daltons

Introduction

Parkinson Disease

Parkinson Disease (PD) is the second most common chronic progressive neurodegenerative disorder with approximately a 2% probability of occurrence during a lifetime. It clinically manifests through uncontrolled tremor, muscular rigidity, bradykinesia, and postural instability coupled with several non-motor symptoms, such as cognitive impairment, autonomic complications, and psychiatric problems (Savitt *et al.,* 2006; Dorsey *et al*., 2007). Approximately 1% of people may be affected by PD by the age of 65 years; this prevalence elevates to 4 to 5% of the population by the age of 85 years (Xiong *et al.,* 2017). About 85% of people have forms of PD without a clearly identified inherited basis, while 5 to 10% of people with Parkinsonism have rare familiar or inherited conditions (Lesage & Brice, 2009). Both broad classes of PD originate from the progressive depletion and death of dopamine-producing nigrostriatal neurons in the *substantia nigra pars compacta* (SNpc) of the midbrain (Fanali *et al*., 2010) and axon terminals projecting to the dorsal striatum (Hornykiewicz, 1962). Alongside nigrostriatal dopaminergic degeneration, most of the non-dopaminergic symptoms of PD get more severe with advancement of age and progression of the disease. A distinctive neuropathological feature of PD is the presence of neuronal inclusions termed Lewy bodies (LB) and of Lewy neurites (LN) due to fibrillar aggregation of phosphorylated α-synuclein, ubiquitin, and other proteins concentrated in susceptible regions of the central nervous system (CNS) and

peripheral autonomic nervous system (Cavallarin *et al.,* 2010; Forno, 1996; Lebouvier *et al*., 2010). Irregular secretion of neurotransmitters, in addition to dopamine, is linked to the motor and non-motor symptoms of PD etiology (Barone, 2010). At present, significant research efforts are involved in the deciphering of the genetic and molecular pathways of neurodegeneration, LB inclusion, and the ubiquitin-proteasome mechanism related to PD by the functional characterization of susceptible PD genes.

Causative and associated genes of PD

The analysis of the potential causes of PD indicates that the disease is a sporadic disorder largely due to a combination of environmental, toxic, and epidemiological factors. Therefore, a basic unified cause of PD has not been established as of yet. However, genes linked to PD have contributed to a better comprehension of the sporadic or idiopathic and the inherited forms of PD through epidemiologic studies (Dawson & Dawson, 2003). Since the discovery of the first PD gene *PARK1/PARK4*, which encodes the protein α-synuclein (Polymeropoulos *et al*., 1997), a considerable number of genes with variants that lead to susceptibility have been added to the list of PD causative genes. Genetic analysis, molecular identification and characterization, neuropathologic investigations, and novel experimental models of PD in both vertebrates and invertebrates illustrate that at least 16 distinct gene loci are responsible for Mendelian forms of PD (Thomas & Beal*,* 2011). The research conducted into PD has found *LRRK2* (Paisán-Ruíz *et al*., 2004), *UCHL1* (Leroy *et al*., 1998), *HTRA2* (Satake *et al*., 2009), *VPS35* (Zimprich *et al*., 2011) including *SNCA*

are autosomal dominant (AD) or gain-of-function form of PD genes. Autosomal recessive (AR) or loss-of-function mutant genes include *parkin or park* (Kitada *et al*., 1998), *PINK1* (Valente *et al.,* 2004), *Dj-1* (Bonifati *et al*., 2003), *GBA* (Aharon-Peretz, *et al.,* 2004), *ATP13A2* (Ramirez *et al*., 2006) and *FBXO7* (Di Fonzo *et al.,* 2009). Of the many candidate genes, the best studied include *SNCA, parkin, PINK1, LRRK2, DJ1* and *VPS35* (Kim & Alcalay, 2017). Either through loss-of-function or gain-of-function, these PD genes (Table 1) are responsible for abnormal protein aggression (LB and LN), alteration of mitochondrial function, oxidative stress, autophagy, mitophagy, lysosomal and Golgi body protein trafficking, protein phosphorylation, and abnormal handling of misfolded proteins. (Krüger, 2004; Eriksen *et al.,* 2005; Thomas & Beal, 2011; Kalinderi *et al.,* 2016;). Studying these genes and the role they play in the progression of the disease has helped to elucidate common pathways in PD pathogenesis.

Drosophila melanogaster **as a model organism for neurodegenerative diseases**

Drosophila melanogaster, commonly known as the "fruit fly" has played an innovative and noteworthy pioneering role in biological research (Staveley, 2012). As a model organism, *Drosophila melanogaster* has been used for the study of animal development, behaviour, neurobiology, and human diseases and disorders, including Parkinson Disease. Easy to culture, with a short life cycle, a non-problematic breeding process, and a small genome size with small chromosome numbers fortify its place in the genetic study (Burdett & Vanden Heuvel, 2004). Although, there is a disparity between

Table 1: List of established genes responsible for familial forms of Parkinson Disease

HGNC symbol	Gene	Inheritance	Probable function	References
PARK1	α-synuclein/ *SNCA*	EOPD AD and sporadic	Presynaptic protein, Lewy body, lipid and vesicle dynamics	(Polymeropolus *et al.,*1997)
PARK2	*Parkin*	Juvenile and AR EOPD and sporadic	Ubiquitin E3 ligase, mitophagy	(Kitada *et al*., 1998)
PARK3	Unknown	LOPD AD	Unknown	
PARK4	*SNCA*	EOPD AD	Presynaptic protein, Lewy body, lipid and vesicle dynamics	(Polymeropolus *et al*., 1997)
PARK5	*UCH-L1*	LOPD AD	Ubiquitin C-terminal hydrolase	(Leroy *et al*., 1998)
PARK6	*PINK1*	AR EOPD	Mitochondrial kinase phosphorylates parkin	(Valente *et al*., 2004)
PARK7	*DJ-1*	AR EOPD	Oxidative stress, Chaperone, Antioxidant	(Bonifati *et al*., 2003)
PARK8	*LRRK2*	LOPD AD and sporadic	Kinase signalling, cytoskeletal dynamics, protein translation	(Paisán-Ruíz *et al*., 2004)
PARK9	*ATP13A2*	Juvenile Kufor–Rakeb syndrome, and EOPD AR	Lysosomal dysregulation, autophagic flux reduction, and mitochondrial clearance. Putative involvement in the oxidative stress response.	(Ramirez *et al*., 2006; Tsunemi *et al.,* 2014)
PARK10	Unknown	PD Risk Factor	Unknown	
PARK11	Unknown	LOPD AD	IGF-1 signalling	
PARK12	Unknown	PD Risk Factor	Unknown	
PARK13	*HTRA2*	AD or risk factor	Mitochondrial serine protease	(Strauss *et al.,* 2005)

PARK14	*PLA2G6*	Juvenile levodopa-responsive dystonia-parkinsonism AR	Phospholipase enzyme. Mutations in *PLA2G6* cause aberrant ER calcium signalling which may impede mitophagy and the oxidative stress response	(Kinghorn *et al*., 2015; Strokin *et al.,* 2012)
PARK15	*FBXO7*	AR EOPD	Ubiquitin E3 ligase, mitochondrial dysfunction	(Di Fonzo *et al*., 2009)
PARK16	*RAB7L1*	Classical PD Risk Factor	Modify Intraneuronal Protein Sorting Vesicles dynamics, autophagy	(MacLeod *et al*., 2013)
PARK17	*VPS35*	Classical PD AD	Intraneuronal protein sorting	(Zimprich *et al.,* 2011)
PARK18	*EIF4G1*	Classical PD AD	Oxidative stress	(Chartier-Harlin *et al*., 2011)
PARK19	*DNAJC6* DnaJ heat shock protein	Juvenile onset, atypical AR PD	Encodes Auxilin, a member of the Hsp40 chaperone family. Works in tandem with *SYNJ1*to recycle synaptic vesicles.	(Edvardson *et al.,* 2012; Ohtsuka & Hata, 2000)
PARK20	*SYNJ1*	Juvenile onset, atypical AR PD	Encodes synaptojanin-1, a phosphoinositide phosphatase. Works with *DNAJC6* to recycle synaptic vesicles.	(Quadri *et al.,* 2013)
PARK21	*DNAJC13*	LOPD AD	Endosomal transport impairment	(Vilariño-Güell *et al*., 2013)

AR-Autosomal recessive, AD-Autosomal dominant, EO-Early onset, LO-Late onset, HGNC-HUGO gene nomenclature committee

human neuronal distribution and fly brains, both have neural function similarity. Compilation of *Drosophila melanogaster* and human genome sequence has revealed that *Drosophila* have an immensely conserved number of genes compared to human (Whitworth *et al.,* 2006). *Drosophila melanogaster* genome encodes 75% orthologues of human disease genes (Marsh *et al.,* 2003). Moreover, it can be 80 to 90%, or more similar in conserved functional domains to human proteins. To construct a neurodegenerative disease model, *Drosophila melanogaster* is a perfect choice because it has a fully active nervous system with differentiation in vision, smell, muscular control, and memory like mammalian organisms.

Modelling Parkinson Disease in *Drosophila melanogaster*

Though the central nervous system of flies is smaller in size compared to higher vertebrates, more than 100,000 neurons mediated by neurotransmitter complexes are well conserved in humans. Approximately 200 dopaminergic neurons (DA) are involved in complex behaviour patterns of flies, such as fertility, developmental time, sleep, and motor control (Neckameyer, 1996; Pandey & Nichols, 2011; Van Swinderen & Andretic, 2011). Studies in this model organism have mapped out important dopaminergic genes whose modification lead to phenotypic expression like PD. Therefore, their mechanisms affect various influential etiologies of PD and highlighting potential treatment strategies (Feany & Bender, 2000). Irrespective of the affected human tissues, the homologues of human genetic disease loci demonstrate selective expression in the *Drosophila* tissues (Chintapalli

et al., 2007), and it is possible to control ectopic expressions of specific genes related to dopaminergic activity in various ways (overexpression or inhibition) by using the UAS-GAL4 system in *Drosophila* (Phelps & Brand, 1998). GAL4 and UAS-target genes are maintained in distinct fly lines: directed expression and responder lines, respectively (Figure 1). The yeast transcriptional activator GAL4 of directed line, under promoter control, directs the transcription of a gene that resides downstream of the upstream activating sequence (UAS) of responder line and directs the expression of that particular gene in a specific tissue, such as eye, muscles, neuron or whole body in a time-specific manner. The target gene is silent in absence of GAL4.When crosses between flies are established, in the progeny the activator factor of GAL4 binds to the enhancer region of the target gene in UAS to induce transcription of the gene. Different types of tissue-specific GAL4 fly lines are utilized in PD modelling, including the motor neuron-specific promoter; D42, the predominately dopaminergic and other neurons-specific promoter; dopa decarboxylase (Ddc), the dopaminergic neuron-specific promoter; tyrosine hydroxylase (TH), and the eye-specific promoter; glass multiple reporter (GMR) (Feany & Bender, 2000; Boto *et al.,* 2014). This technique is widely used and convenient for selective expression or knockdown of specific genes in various lines of flies.

The compound eye of an adult *Drosophila melanogaster* is composed of approximately 800 hexagonal visual units called ommatidia (Neriec & Desplan, 2016). Each ommatidium consists of eight photoreceptor neurons, and the total visual system contains about 150,000 neurons and glia cells (Chiang *et al*., 2011). Neurodegeneration can be examined by observing the ommatidium, inter-ommatidial bristles, eye shape,

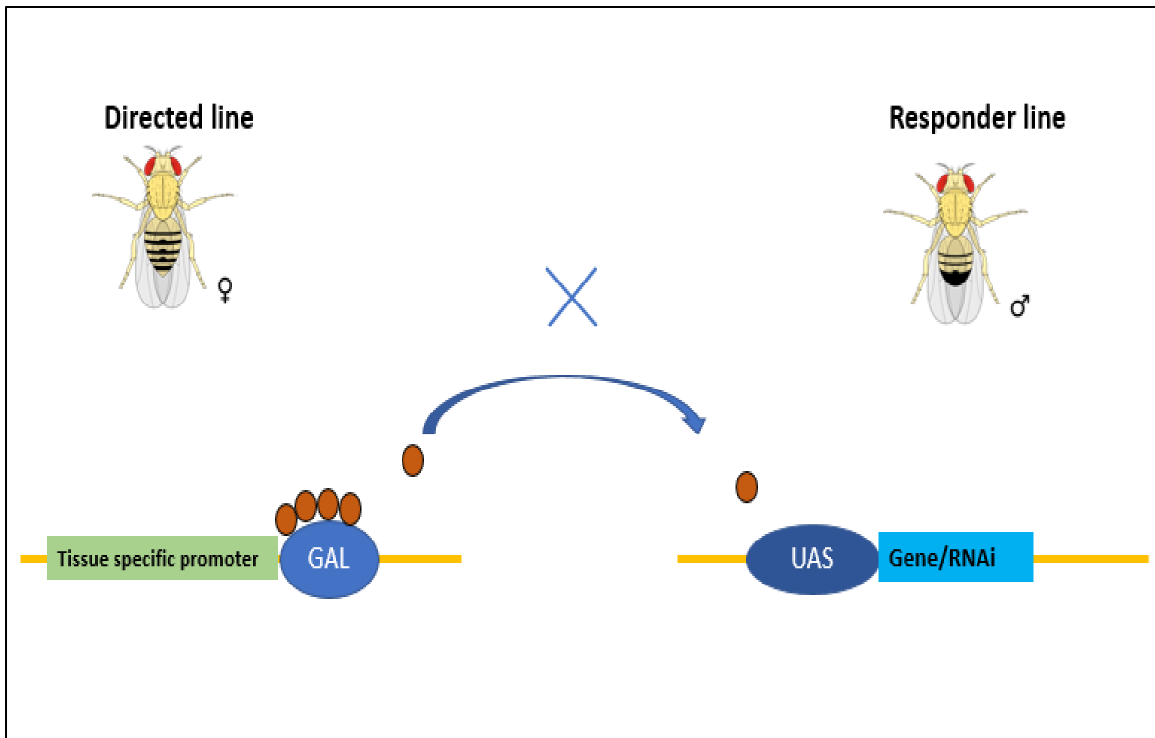

Figure 1**. The GAL4-UAS directed expression system.**

Maintained in two distinct lines of flies, in the critical class of progeny the yeast derived transcriptional activator GAL4 of the directed expression line, under a tissue specific promoter control, binds to the upstream activating sequence or UAS of desired gene in responder line and drives the transcription of the target gene.

disruption, ratio, and lobe, as the development and continued maintenance of all neurons are related to the functional structure of the adult eye. Analysis of altered eyes has cast a light into the field of cellular mechanisms, signalling transduction and transcription, cell proliferation, growth, and apoptosis (Kumar, 2012). Therefore, various types of neuronal gene expression in different tissues of *Drosophila melanogaster* allows modelling of major aspects of PD.

Models of PD

The α-synuclein model

The α-synuclein (*SNCA*/*PARK1/PARK4*) is known as the first identified gene in rare and severe familial forms of PD pathology, which encodes a small and soluble presynaptic nerve terminal protein (Polymeropolus *et al.,*1997). This gene represents one of the most abundant protein components found in Lewy bodies, and it is believed to play an important role in the progression of PD by creating insoluble fibril depositions in the brain. While *Drosophila melanogaster* lacks a clear *α-synuclein* orthologue, expression of the wild human type and two mutant forms of *α-synuclein*, A30P and A53T are responsible for phenotypic features that model autosomal-dominant PD in flies. Additional *SNCA* triplication and duplication mutations, this model can reproduce similar key features of PD, such as, adult-onset degeneration and loss of DA neurons, retinal degeneration, aggregation of proteinaceous α-synuclein inclusions, and locomotor defects (Botella *et al.,* 2009). In *Drosophila,* expression of both α-synuclein normal and mutant forms generate adult-onset degeneration of dopaminergic neurons, filamentous intraneuronal aggregation containing α-synuclein, and locomotor disorder. The *Drosophila* model thus recapitulates the essential features of PD, which makes it a powerful genetic approach to this disease (Feany & Bender, 2000). Auluck and colleagues (Auluck *et al.*, 2002) argued that dopaminergic neuronal loss due to α-synuclein in flies could be prohibited by the overexpression of the chaperone protein Hsp70, thereby indicating the regulatory role of this chaperone in α-synuclein toxicity. Another investigation showed that geldanamycin-treated flies had a reduced stress response to elevated chaperone activity, which ultimately protects against

α-synuclein induced neurotoxicity (Auluck *et al*., 2005). However, the whole mount immunohistochemistry analysis of α-synuclein fly models proved that there was no loss of dopaminergic neuronal cells in fly brains (Pesah *at el*., 2004). Maybe some environmental factors influenced this experiment and more analysis is needed to justify and clarify this issue. ROS causes damage of intracellular components (lipids, proteins, and DNA in post-mortem PD brain) and enhances apoptotic cell death, both of which are common in autopsy tissue from the brains of PD patients. In rat mutants, a model of *SNCA* showed that, mis-folding of alpha-synuclein oligomers induced ROS and neuronal toxicity by production of aberrant free metal ions. (Deas *et al*., 2016). Overexpression of *Rab1*, the mammalian *YPT1* homologue, suppressed α-synuclein-induced dopaminergic neuron loss in animal models of PD (Cooper *et al.,* 2006). Sirtuin 2 inhibited *SIRT2* via interfering RNA, and rescued α-synuclein toxicity and changed protein inclusion structure of α-synuclein in a cellular model of PD. This inhibitor protected against dopaminergic cell death both in vitro and in a *Drosophila* model of PD (Outeiro *et al*., 2007). Co-expression of *park* can suppress phenotypes caused by the expression of mutant α*-synuclein*, such as in the developing eye, where *park* reduces retinal degeneration. When co-expressed in the dopaminergic neurons, the ability to climb was improved in flies (Haywood & Staveley, 2006; van der Merwe *at el*., 2015). Further exploration of the interaction of α-synuclein with other proteins can open new therapeutic solutions of PD. These variations show the complexity of biological systems that are modified by *α-synuclein,* which plays an important role in elucidating intricate pathways of PD.

The *parkin* model

The *parkin* gene encodes a cytosolic ubiquitin isopeptide E3 ligase that is 465 amino acid residues in length; it contains an N-terminal ubiquitin-like domain (Ubl), and four Zn-coordinating really interesting new gene (RING)-like domains: RING0, RING1, IBR (in-between RING), and RING2. (Shimura *et al*., 2000; Finney *et al*., 2003; Seirafi *et al.,* 2015). RBR (RING-between-RING) domains of parkin are involved in substrate identification, binding ubiquitin interacting motif (UIM) domains, degradation of misfolded protein by ubiquitin proteasome system (UPS), and the regulation of cellular parkin levels of two functional sites (being the binding and catalytic sites). It ubiquitinates multiple numbers of cytosolic and outer mitochondrial membrane proteins upon mitochondrial depolarization. *Parkin* plays a central role in the cascade reaction, where E3 ubiquitin-ligating enzyme conjugates with E1 ubiquitin-activating enzymes and E2 ubiquitin-conjugating enzymes of the ubiquitin proteasome system (UPS) to ubiquitinate misfolded or agglomerated proteins. These proteins later target impaired or unwanted proteins for degradation by the 26S proteasome (Kazlauskaite *et al*., 2014). More than 170 pathogenic PD mutations have been determined throughout parkin domains, causing *PARK2* protein loss of function for each of the individual domains (Pickrell & Youle, 2015). The protein products of *parkin* promotes the UPS for the accumulation of impaired mitochondria which enhances toxic reactive oxygen species (ROS) and contributes to the degradation of dysfunctional mitochondria by autophagy and neuronal cell death in PD (Narendra *et al.,* 2008; Chan *et al*., 2011; Ashrafi *et al.,* 2014). Numerous studies over the years have proven that protein product of *PINK1* and *parkin* follow the same

biochemical pathways to clear damaged mitochondria (Narendra *et al.,* 2012). *PINK1* acts upstream of *parkin,* which accumulates first on dysfunctional mitochondria. Then its kinase activity phosphorylates *parkin* (Kane *et al*., 2014). Upon activation by *PINK1*, *parkin* ubiquitinates the damaged mitochondria through the modification of numerous cytosolic and outer mitochondrial membrane (OMM) proteins such as Mitofusin or Miro to instigate the removal of mitochondria by mitophagy. In this pathway, the mitochondrial kinase *PINK1* senses mitochondrial fidelity and recruits *parkin* selectively to mitochondria that lose membrane potential (Sauve *et al*., 2015; Pickrell & Youla, 2014). These two proteins function together in a mitochondrial quality control pathway whereby *PINK1* accumulates on damaged mitochondria and activates *parkin* to induce mitophagy.

Several mutations, such as point mutation and deletion are related to *parkin* (PARK2), have provided new insights into the survival of dopaminergic neurons in both sporadic and familial models of PD in *Drosophila*. Alongside degeneration and loss of DA neurons in the adult brain, these mutant flies exhibit apoptotic flight muscle degeneration and locomotor defects from mitochondrial dysfunction, hypersensitivity to reactive oxidation stress and environmental stress, reduced life span, male sterility, developmental delay, and decreases in cell count and size (Greene *et al*., 2003; Hattori & Mizuno, 2004; Pesah, *.et al.,* 2005). Loss-of-function mutations in *parkin* produced high amounts of free radicals, possibly in high energy-dependent cell groups with reduced cell size (Pesah, *et al*., 2005). Loss-of-function mutations of the *glutathione S-transferase S1* (*GstS1*) gene in flies display a degeneration of specific dopaminergic neurons in the brain but overexpression of *GstS1* in DA neurons reduces neurodegeneration in *parkin* mutants

(Whitworth *et al.,* 2005). The Pael receptor, a transmembrane polypeptide, interacts with *parkin* and leads to unfolded and accumulated protein-induced selective neuronal death in autosomal recessive juvenile Parkinsonism (Imai *et al.,* 2001). Moreover, the overexpression of a human *parkin* mutant R275W in *Drosophila* resulted in an age-dependent degeneration of specific DA neuronal clusters, concomitant locomotor deficits that were exacerbated with age, and mitochondrial abnormalities in the flight muscles. The above-mentioned defects caused by the expression of human R275W *parkin* are highly similar to those directed by the loss of *parkin* in *park* null flies (Wang *et al.,* 2007). The findings of *park* mutants in flies are still going on and have reiterated the significance of this model organism in revealing the pathology of human PD.

The *PINK1* model

The *PINK1* gene encodes a 581 amino acid protein consisting of an N-terminal mitochondrial targeting motif and a conserved kinase domain (serine/threonine kinase) homologous to the Ca^{2+}/calmodulin family (Valente *et al*., 2004). Several nonsense and missense mutations in *PINK1* cause early onset autosomal recessive (EOAR) parkinsonism with atypical features to clinical presentations that differentiate from sporadic late-onset PD (Silvestri *et al*., 2005; Clarimón *et al*., 2006; Muqit *et al*., 2006;). A lack of *PINK1* genes results in an age-dependent loss of neuronal viability and increased sensitivity to oxidative stress and stress-induced mitochondrial apoptosis. Dysregulation of mitochondria is implicated due to the presence of lowered mitochondrial membrane potential (Wood-Kaczmar *et al*., 2008). *PINK1* encodes serine-threonine kinase which

regulates a set of substrates prone to protein agglomeration and plays an important role in mitochondrial homeostasis. Both mitochondrial dysfunction and kinase pathways regulated by *PINK1* play important roles in neurodegeneration. Drosophila *PINK1* is found to localize in the mitochondrial membrane like human *PINK1*. The pathogenic role of *PINK1* in maintaining mitochondrial integrity has established with mutational analysis of the *Drosophila* orthologue by transposon-mediated mutagenesis and RNAi. Loss of *PINK1* causes degeneration of apoptotic flight muscles, defective spermatid formation, DA neuronal death, locomotor deficits, and oxidative stress in flies (Greene, *et al.,* 2003). These physical features are associated with defective mitochondrial morphology and disruptions, and share prominent phenotypic similarities with *parkin* (*park*) mutants. The *park* protein product functions downstream of *PINK1*: its transgenic expression in flies markedly improved all *PINK1* loss-of-function phenotypes, but not vice versa (Clark *et al*., 2006; Gandhi *et al.,* 2006; Park *et al*., 2006; Banerjee *et at.,* 2009). All of these observations support the notion that mitochondrial dysfunction is a vital contributing factor to DA neuron loss in patients with altered *PINK1* function.

The *DJ-1* model

DJ-1 belongs to the ThiJ/Pfpl protein superfamily and acts as a suppressor of PTEN function. *DJ-1* encodes an 189 amino acid protein that is shown to have a functional domain resemblance to proteases, kinases and heat shock proteins (Thomas & Beal, 2011). This gene controls cell death and survival through multiple cellular functions. It acts as a redox-sensitive molecular chaperone in an oxidative cytoplasmic environments, operates an

antioxidant action for ROS, stabilizes antioxidant transcriptional master regulator nuclear factor Nrf-2, transforms cells in cooperation with H-*ras,* and androgen receptor signalling (Da Costa, 2007; Bonifati *et al*., 2003; Kim, R. H. *et al*., 2005; Menzies *et al.,* 2005; Abou-Sleiman *et al.,* 2006). Mutations in the C-terminal helix of *DJ-1* that alter antioxidant function have been identified in various PD patients which associated with autosomal EOPD (Bonifati *et al*., 2003; Tao & Tong, 2003). *Drosophila melanogaster* homologues of *DJ-1* play critical roles in the survival of dopaminergic neurons and response to oxidative stress. Two homologues of *DJ-1* in *Drosophila* are *DJ-1α* and *DJ-1β,* which show different results in the *DJ-1* loss-of-function model. *DJ-1β* is expressed ubiquitously, while *DJ-1α* is expressed only in the male germline. Knockout of both homologues generates fertile flies with normal lifespan, but they are susceptible to environmental factors such as paraquat (1,1'-Dimethyl-4,4'-bipyridinium dichloride) and rotenone. These factors are related to PD in humans and the resultant loss of *DJ-1β* protein is responsible to increase oxidative stress (Meulener *et al.,* 2005). Another experiment shows that loss-of-function *DJ-1β* mutants increase the survival of dopaminergic neurons and resistance to the effect of paraquat stress, but are very susceptible to hydrogen peroxide treatment. Upregulation of *DJ-1α* expression in the brain of the *DJ-1β* mutant resulted in a compensatory effect for paraquet, rotenone, and hydrogen peroxide sensitivity (Menzies *et al.*, 2005). RNA interference (RNAi) mediated inhibition of *DJ-1α* results in cellular aggregation of reactive oxygen species, acute sensitivity to oxidative stress, degeneration of dopaminergic neurons, and impairment of PI3K/Akt signalling (*Yang et al*., 2005). Expression of only the PTEN transgene in the eye imaginal disc resulted in a 30% decrease

in eye size with reduced cell viability. However, the co-expression of PTEN and *DJ-1* suppressed the PTEN-induced eye phenotype and rescued cell death (Kim *et al*., 2005; Kim, 2005). Application of various dietary antioxidants, such as vitamin E, melatonin, and vitamin C extends the lifespan of mutant flies (Kumar *et al.,* 2017). The same result is observed in spirulina feeder flies along with improvement of locomotion ability. There are many biological functions described by which the loss of *DJ-1* function protects against neuronal demise, but it seems most likely that the major role of *DJ-1* is in response to oxidative stress to maintain mitochondrial integrity.

The *LRRK2* model

Leucine-rich repeat kinase (*LRRK2)* is a large 2527 amino acid long, multidomain protein, which contains Ras of complex (Roc), putative serine/threonine kinase, GTPase domains, C-terminal of Roc (COR), kinase domain of MAPK, and leucine-rich repeat domain (LRR) (Lee *et al.,* 2001). These multifunctional domains are implicated in various cellular modifications including alternation of enzymatic phosphorylation, cellular transformation, and vesicle trafficking, and stimulation of stress-activated kinase that leads to neuronal degeneration. The *GAL4*/*UAS* system of mediated transgenic expression of either wild-type human *LRRK2* or *LRRK2*-G2019S in the photoreceptor cells caused retinal degeneration of *Drosophila*. A similar expression in neurons generated an adult-onset selective loss of dopaminergic neurons, locomotor dysfunction, and premature mortality (Liu, Z. *et al*., 2008). Wild-types, as well as transgenic G2019S-LRRK2 rats, have attenuated α-synuclein induced dopaminergic neurodegeneration and inflammation by

LRRK2 kinase inhibition (Daher *et al*., 2015). The inhibition of LRRK2 activity can have a disease-modifying effect (Paisán-Ruíz *et al*., 2004). In double transgenic flies, various phenotypes of the eye and dopaminergic survival changed in a complex fashion through a concomitant expression of *PINK1, DJ-1,* or *parkin* (Venderova *et al.,* 2009). *LRRK2* motor neuron mediated proboscis extension response (PER) in *Drosophila* shows slower movements, absence of movement altogether, increased tremor, and changes in neural signalling (Cording *et al.,* 2017). All this evidence suggests a genetic interaction of *LRRK2* between these PD-relevant genes.

The role of the mitochondria in PD

As the main functional organelle responsible for energy metabolism, mitochondria regulate several cellular processes, most significantly oxidative stress, calcium balance, substrate binding, and apoptosis. Disrupting mitochondrial dynamics ultimately contributes to neuronal cell death and neurodegenerative diseases. Complexes I to V of the electron transport chain within mitochondria transfer the electrons to the respiratory chain across the inner mitochondrial membrane by the electrochemical membrane potential (proton-motive force). ROS is produced, in this oxidative phosphorylation process. (Starkov, 2008; Murphy, 2009). An elevated level of mitochondrial ROS or defective ROS elimination promotes the oxidative damage of mtDNA, disturbs proteins and lipids signalling pathways, increases free hydroxyl radicals, and bioenergetic failure, which can perturb the whole process of mitochondrial ageing, fission and fusion. Analysis of mitochondria from the frontal cortex of PD patients demonstrated that reactive subunits of

complex I are oxidatively damaged, corroborating the mitochondrial effect with PD pathology (Winklhofer & Haass, 2010). Further explanation is required about which subunits are affected for better understanding. Inhibitors of complex I such as 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydrodropyridine (MPTP), pyridaben, rotenone, trichloroethylene, and fenpyroximate induce dopaminergic neuronal demise in flies, humans and rodents. (Chaturvedi & Beal, 2008). These toxins that affect mitochondria in the flow of the electron transport system cause an increment in the mitochondrial membrane permeability and increase aberrant reactive oxygen species (ROS) (Borland *et al*., 2008; Bose & Beal, 2016; Moon & Paek, 2015). Structural changes in complex I lead to dopaminergic neurodegeneration, making these dopaminergic neurons more susceptible to neurotoxins (Perier *et al.,* 2010). Mitochondrial DNA (mtDNA) is a double-stranded small circular genome which replicates independently, and contains 37 genes, which encode many proteins. Inhibition of midbrain dopaminergic neurons causes reduced mtDNA expression, respiratory chain deficiency and neuronal cell death, leading to progressive, L-dopa-responsive impairment of motor functions in rodents (Winklhofer & Haass, 2010). Neurons are required to act promptly in responding to bioenergetic demand. The electrochemical potential of presynaptic and postsynaptic sites in neurons depends on the efficient transport of mitochondria. Thus, it is assumed that mitochondrial modifications can promote neuronal dysfunction and degeneration (Exner *et al.,* 2012). Evidence from multiple studies indicates that PD-associated genes and various mutations directly or indirectly affect mitochondrial integrity (morphology, function, and dynamics), thereby providing a mutual link to pathophysiological alterations observed in sporadic and familial PD (Abou-Sleiman

et al., 2006). Both wild type and mutant *SNCA* mouse models exhibit mitochondrial abnormalities: degenerating mitochondria with accumulated SNCA, complex I and IV impairment, mtDNA damage, enhanced mitochondrial Ca^{2+}, and oxidative modification, which are prevalent in mitochondria from striatum and substantia nigra (SN) of PD patients (Subramaniam *et al.,* 2014). Both genes *parkin* and *PINK1* modify mitochondrial biogenesis (Henchcliffe & Beal, 2008). A small portion of DJ-1 protein resides in the mitochondrial matrix, which inhibition in the motochondria of the fly and mouse showed decreased mitochondrial DNA levels, sensitivity to MPTP-induced loss of dopaminergic neurons, and reduced complex I activity (Lev *et al.,* 2006). Mutation in the GTPase kinase domain of *LRRK2* caused mitochondrial disintegration and fusion reduction alongside defects in intracellular protein distribution (Martin *et al.,* 2014). HtrA serine peptidase 2 (*HTRA2*) controls mitochondrial membrane permeability during programmed cell death and changes mitochondrial morphology via OS vulnerability (Li, B. *et al*., 2010). All (*SNCA, PINK1, Parkin, DJ-1 LRRK,* and *HTRA2*) major genes of familial PD show connection to mitochondrial paradigms associated with the disease including anomalies of the mitochondrial ETS, mutated protein deposition, age-dependent damage to mtDNA, impaired calcium homeostasis, altered mitochondrial morphology, and biogenesis. Recently added novel gene mutations, and their impact on mitochondrial functions, have further reinforced the relevance of mitochondrial abnormalities in PD disease pathogenesis.

The ubiquitin-proteasome system (UPS) in PD

The UPS is the main pathway to carry out a highly selective degradation of short-lived, small, intracellular misfolded as well as ubiquitinated proteins in the nuclear and cytoplasmic compartments (Ross *et al.,* 2015). This system involves two consecutive steps: ubiquitylation-binding of ubiquitin to a substrate protein through an enzymatic cascade and proteasomal degradation. Multiple rounds of ubiquitylation lead to the formation of a polyubiquitin chain, which can provide a signal for degradation by the 26S proteasome, a multi-protein complex consisting of a 20S core particle and 19S regulatory particles. The proteasome unfolds substrates, and only the unfolded ubiquitinated polypeptide chains can pass through the inner channel of the proteasome barrel (Dantuma & Bott, 2014). The poly-ubiquitin chain is synthesized by a cascade reaction consisting of three enzymes: E1 (ubiquitin-activating enzyme) binds to a ubiquitin molecule, E2 (ubiquitin-conjugating enzyme) receives ubiquitin from E1, and E3 (ubiquitin-ligase enzyme) carries out proteins recruitment relied on substrate specificity (Hochstrasser, 1996). Targeting of the vulnerable proteins by ubiquitin, impaired function of the UPS, and the resultant accumulation of misfolded proteins have been strongly implicated in the pathogenesis of PD. This, in combination with increased oxidative stress, leads to the death of dopaminergic neurons, thereby affecting cell survival. α-synuclein, the major component of LBs in the brain is degraded by the 26S proteasome, and mutated *α-synuclein* instigates the formation of abnormal branches that directly interact with the 20S core of the proteasome to inhibit its proteolytic function (Heo & Rutter, 2011). Parkin mutations in AR-JP shows reduced ubiquitin-ligase enzymatic activity in the substantia nigra, which affects the UPS

negatively and leads to the PD neurodegeneration (Ebrahimi *et al.,* 2012). *UCH-L1* is an early onset AD PD susceptibility gene that is involved in regulation of substrates and releases ubiquitin from cellular amines. Dimerized UCH-L1 modifies α-synuclein ubiquitination, resulting in PD pathology (Atkin & Paulson, 2014). Another PD susceptible gene, the *FBXO7* constitutes one of the four subunits of the ubiquitin ligase complex that is implicated in the UPS pathway and this gene product has been found in Lewy bodies of PD patients (Zhao *et al.,* 2013). All of these observations suggest a large degree of inter dependable correlation between UPS and mutations in the PD genes which ultimately leads to the degeneration of DA neurons.

The endoplasmic reticulum and Golgi body link to PD

The endoplasmic reticulum (ER) is a cytoplasmic organelle that maintains protein homeostasis in the cell, which is a complicated stepwise process starting from synthesis to gradual folding, assembly, transport, and degradation of proteins. Golgi body is the packaging centre of the cell, which after receiving protein vesicles from the ER, proceeds to further modification: glycosylation, phosphorylation, proteolytic cleavage and trafficking to the destination (Bexiga & Simpson, 2013). ER and Golgi related dysfunctions hinder the anterograde and retrograde transport of proteins and lead to the accumulation of misfolded proteins, which activates the unfolded protein response (UPR) for cell survival (Wang *et al*., 2016). Signs of altered protein deposition in the ER lumen are visible in the post-mortem tissue of human and animal models of PD. Accumulation of ER-associated degradation substrates, and ER stress was identified as an early pathologic phenotype in

neurons developed from induced pluripotent stem cells of patients having α- synuclein mutations (Chung *et al.,* 2013). α-Synulcein inhibits the ATF6 activating transcription factor and ATF6 incorporation into coat protein complex (COP)II vesicles, resulting in an aberrant unfolded protein response (Credle *et al.,* 2015). It indirectly impairs ER-Golgi protein transport, which enhances further ER stress and apoptosis (Wang *et al*., 2016). Mutations in the E3 ubiquitin ligase parkin cause the aggregation of α-synuclein owing to reduced ubiquitin ligase activity, which ultimately affects both ER and Golgi body transport (Mercado *et al*., 2016). In the yeast model, the overexpression of the ER-Golgi trafficking genes *YPT1, YKT6, BRE5, UBP3*, and *ERV29* suppresses α-synuclein induced toxicity by promoting forward ER-Golgi transport (Cooper *et al*., 2006). The fragmentation of the Golgi body is correlated with fluctuations in levels of RAB1, RAB2, RAB8 and the SNARE protein syntaxin-5 (STX5), but the accumulation of the pre-synaptic protein α-synuclein could be alleviated by *RAB1* and *RAB8* overexpression and *RAB2* and *STX5* depletion in the affected cell (Bexiga & Simpson, 2013; Rendón *et al.* , 2013). All of these proteins are related to the early secretory pathway machinery, and they indicate that the ER dysfunctional trafficking, and Golgi fragmentation, occurred before α-synuclein aggregation, and activates the progressive formation of the inclusion bodies.

The endosomal-lysosomal regulation in PD

The endosomal-lysosomal system consists of interconvertible membranous compartments, namely early endosomes, recycling endosomes, late endosomes, and the

lysosome. The lysosome represents the common degradative endpoint at which the endosomal and autophagic pathways converge. The organization and functions of membrane-bound organelles dynamics (endocytic trafficking) which affect protein (α-synuclein) accumulation (Schapira & Jenner, 2011), and degradation. Recently, rising evidence has suggested that the abnormalities in the physiological processes of endosomal-lysosomal maturation, acidification, and sorting systems during the endocytic transport/trafficking, especially autophagy, is directly inducive to the neurological malfunctions, represented by PD, and Lewy body dementia (LBD) (Hu *et al*., 2015). Macroautophagy and chaperone-mediated autophagy (CMA) are the two main processes of autophagy lysosomal pathway (ALP) and both regulate protein degradation (wild type α-synuclein is degraded by the CMA) upon the accumulation of misfolded, damaged, or unnecessary proteins. Autophagic dysregulation causes the aggregation of abnormal proteins or damaged organelles, a characteristic of α-synuclein induced PD. Genes pertaining to recessive PD, such as *PINK1* and *parkin* (PINK2), combinedly interact in the process of mitophagy, whereby defective mitochondria are selectively engulfed by macroautophagy (Xilouri & Stefanis, 2011). Heterozygous mutations in the lysosomal enzyme glucocerebrosidase (*GBA1*) increase the risk of PD. *LRRK2* interacts with and disrupts endocytosis, and late endosome-lysosome trafficking. Both the G-associated kinase, *GAK,* and *RAB7L1* contribute to trans-Golgi complex formation and cause an impairment in endo-lysosomal trafficking and Golgi apparatus sorting (Perrett *et al*., 2015). The PD-causing D620N mutation of *VPS35* in transgenic flies caused mobility impairments, shortened lifespans, bristles loss, eye disorganization, and increased

sensitivity to PD-linked environmental toxins (rotenone) (Wang *et al*., 2014). In *Drosophila* and mammalian cells, the D620N mutation in *VPS35* resulted in α-synuclein accumulation in late endosomes/lysosomes, probably due to an alteration in the trafficking of lysosomal protease, cathepsin D, and endosomal disruptions which degrade α-synuclein (Follett *et al*., 2014; Miura *et al*., 2014). This mutation also reduces endosomal sorting WASH (Wiskott–Aldrich syndrome protein and SCAR homologue) complex recruitment to early endosomes, and, therefore, indicates an association with perturbing endosomal/lysosomal trafficking (Zavodszky *et al*., 2014). Like *VPS35*, mutations in *DNAJC13* are related to the WASH complex inhibition to *FAM21* protein binding. Endosomal tubules are formed by sorting nexin dimer-SNX, *DNAJC13* may regulate the localization of the WASH complex and SNX dimer, as its suppression causes an increase in highly branched endosomal tubules (Freeman, 1996). Interestingly, all the genes mentioned above encode proteins involved in the endo-lysosomal system, and directly or indirectly associated with higher risk or susceptibility to PD. This strongly supports the notion that endosomes play a pivotal role in the disease.

Gene of interest

Large-scale research efforts, genome-wide association studies (GWAS), transgenic animal designs, next-generation sequencing (NGS), and exome sequencing implementation, have undeniably accelerated the identification of new phenotype-associated genes for sporadic and monogenic PD (Kalinderi *et al*., 2016). Whole genome

expression microarrays, combined with expression quantitative trait loci (eQTL) analysis showed 7 of 424 genes have the presence of prominent eQTLs in various brain regions, including the basal ganglia, which provides insight in the PD pathway (Murthy & Ramachandra, 2017). Recently, through the integration of cell-based and model organism functional screens, it has been conferred (Jansen *et al*., 2017) that there are potential links between the 27 de novo autosomal recessive candidate genes and the well-established mechanisms of PD susceptibility and pathogenesis, including mitochondrial dynamics and α-synuclein-mediated toxicity. There is an illustration of two gene-association networks, the first one centered around *FBXO7* and *LRRK2*, the second one centered around *SNCA, PINK1, PARK2, PARK7, ATP13A2, and GBA. UHRF1BP1L* is one of the five functionally validated genes which shows a strong interaction with PD genes of the second network (*SNCA, PINK1, PARK2, PARK7, ATP13A2, and GBA).*

UHRF1BP1L [Ubiquitin-like PHD finger and Ring finger domain-containing protein 1(UHRF1) Binding Protein 1 Like] is a protein-coding gene. Alias symbols for this protein are KIAA 0701 and syntaxin 6-interacting protein (SHIP164). KIAA 0701 was first named during the prediction of the coding sequences of unidentified human genes from cDNA libraries of the human brain (Ishikawa *et al*., 1998). This gene belongs to the family UHRF1- binding protein-1 like and the only sequence paralogue of this gene is *UHRF1BP1*. UHRF1 is a biomarker of tumorigenesis that contains a UBL methylated H3 histone domain, PHD domain, SRA domain, and Ring Finger with ubiquitin E3 ligase activity domain (Bronner *et al*., 2007). This protein is localized in the cytoplasm, cytosol, and early endosomes. This protein has two isoforms: isoform a is a 1464 amino acid

sequence and isoform b, a short chain of 522 amino acid sequence with a presumed molecular mass of 164kDa and 59kDa, respectively. The N-terminal (Chorein-N or Vps13 domain) of UHRF1BP1L shows similarity with the N-terminus of yeast Vps13p and human VPS13 (human has four *VPS13* genes) which is a full-length transmembrane protein with a role in vesicle-mediated sorting from endosome to Golgi body (Redding *et al*., 1996; Velayos *et al*., 2004). Chorein protein is encoded by the *VPS13A* gene, whose loss-of-function mutations are responsible for the neurodegenerative disorder, Chorea acanthocytosis, and results in the demise of striatal neurons. This protein is visible in dense-core vesicles of the neurite terminal in rat PC12 cells (a cell line derived from adrenal medulla), which contain dopamine (Hayashi *et al*., 2012; Tomemori *et al*., 2005). Taken together, this indicates that the Chorein-N domain might be involved in intracellular protein transport. UHRF1BP1L has been predicted to have a small coiled-coil region at the C-terminus. Two domains between the N and C- terminals are inosine-5′- monophosphate (IMP) dehydrogenase/guanosine monophosphate (GMP) reductase (ID/GR), and fibronectin type III-like fold (FNIII) (Otto *et al*., 2010). IMP dehydrogenase of the ID/GR domain has phosphate binding site and involved in the synthesis of guanosine nucleotide, and the deamination of GMP to produce IMP is catalyzed by GMP reductase (Smith *et al*., 2016; Zhang et *al*., 1999). FNIII is highly stable, encoded by 90 amino acids in human with an immunoglobulin (Ig)-like fold but it lacks internal disulfide bridges, which have been implicated in cell adhesion, elasticity, and membrane receptors (Shah *et al*., 2017). Domains attached to Ig have been found to beuseful for diagnostic and therapeutic purposes due to their high penetration efficiency into cells and conjugation ability to the therapeutic

drug (Jacobs & O'neil, 2018). Considering the above functionally significant domains of *UHRF1BP1L*, further analysis is important to uncover the molecular therapeutic aspects of PD.

Protein trafficking role of *UHRF1BP1L*

Membrane trafficking of proteins between organelles is mediated by vesicles that bud from one membranous compartment and fuse with a target compartment. The SNARE proteins play a crucial role in various stages of trafficking such as budding, transport, tethering, and fusion to target organelles (Brandhorst *et al*., 2006). In GST pull-down assays, followed by antibody regulated co-immunoprecipitation, the N-terminally placed Habc domain of Syntaxin 6 (SNARE protein) interacts with the predicted C -terminal coiled-coil domain of SHIP164 or *UHRF1BP1L***,** which reflected the same interaction in yeast two-hybrid analysis. Correlated light and electron microscopy showed co-overexpression of both *Stx-6* and *UHRF1BP1L* in transfected cells (human embryonic kidney 293 cells) produce an excessive tubulation in the membranous structure of the early endosomes. All four (*VPS51, VPS52, VPS53*, and *VPS54*) genes of the GARP (Golgi-associated retrograde protein) complex work together in the retrograde transport of protein from endosome to Golgi, which is required for the recycling of intracellular membrane proteins and for vacuolar protein sorting (Otto *et al*., 2010). Interestingly, mutations in *VPS35*, a component of the retromer complex, which is involved in endosomes to the trans-Golgi network retrograde transport of protein, is associated with the late-onset PD (Chesi

et al., 2012; Vilariño-Güell *et al*., 2013; Zimprich *et al*., 2011). As well, *UHRF1BP1L* interacts with subunits (Vps52 and Rab proteins) of the Golgi-associated retrograde protein (GARP) and functions in membrane trafficking through the early and late endosomal system (Otto *et al*., 2010). The protein products of *UHRF1BP1L* have similarity in structure with Vps13 (Vacuolar protein sorting-associated protein 13) in the N-terminus and both proteins have a C-terminal coiled-coil region. The N-terminal portions of VPS13A and VPS13C act as lipid transporter between the endoplasmic reticulum and other membranous organelles. The dysfunction of lipid homeostasis due to the mutation of *VPS13C* likely shows clinical manifestations of patients with parkinsonism (Kumar *et al*., 2018) . The Chorein-N domain has been noted in the UHRF1BP1L protein (Mizuno *et al*., 2007), and its presence likely indicates that it has lipid transport modules similar to Vps13. Moreover, this gene shows involvement in protein trafficking from endosome to Golgi Body. This fortifies its potential role in intracellular movement and disposal of alpha-synuclein and ubiquitin protein of PD.

Mitochondrial dysfunction and *UHRF1BP1L*

In both Mendelian and sporadic forms of PD, especially those that result in early onset of the disease, mitochondrial defects are generated by mutations in PD-related genes and contribute to the mitochondrial protein regulation, ETS, oxidative stress, mitophagy, and the fusion or fission of mitochondria (Haelterman *et al*., 2014; Pickrell & Youle, 2015). In mitochondrial mechanisms and functional control, *PINK1(PARK6), parkin (PARK2)*

and Dj-1(PARK7) have impacts that are probably related in creating oxidative stress in neuronal cells (Banerjee *et al.,* 2009; Cookson, 2012). Specifically, *PINK1* (mitochondrial kinase) and *parkin* (E3 ubiquitin ligase) act in combination to remove damaged mitochondria by mitophagy (Rüb *et al.,* 2017). Moreover, aggregation of α-synuclein (*SNCA*), a prominent factor of Lewy bodies accumulation in PD, has induced mitochondrial fragmentation (Kamp *et al.*, 2010). The interest gene *UHRF1PB1L* is significantly co-expressed with Mendelian genes including both *PINK1* and *SNCA* in the *subtantia nigra* of the brain, and the silencing of *UHRF1BP1L* causes alternation of mitochondrial morphology, and mitochondrial numbers reduction. In addition, *UHRF1PB1L* shares proteins domains with VPS13 and VPS13C, and has been linked to reduced mitochondrial membrane potential, mitochondrial fractionation, increased respiration rates, and aggravated *parkin/PINK1* system mitophagy in Parkinsonism with AR (Lesage *et al.,* 2016). Neuronal disorders leukodystrophy and hypomyelination 6 are associated with defects in this gene. Moreover, *UHRF1BP1L* was mentioned as a candidate gene for the most common ocular disorder myopia, and another uncommon skin condition disseminated superficial porokeratosis (Hawthorne *et al.,* 2013). Considering its relationship to other neurological diseases, and mitochondrial vulnerability to AR PD, it is necessary to characterize the putative AR PD gene *UHRF1BP1L* to understand its functional influence on other PD genes and pathology.

CG34126 gene in *Drosophila melanogaster*

As described in this thesis, *CG34126* (Fly base ID FBgn0083962) is the homologue gene of *UHRF1BP1L* in *D. melanogaster,* which is identified in chromosome 2L. The protein encoded by the gene shares its domains with the UHRF1BP1L and the N- terminal domain of the vacuolar protein sorting-associated protein 13 (Jansen *et al.,* 2017). The *CG34126* gene remains to be elaborately analyzed in *Drosophila* for PD, but as a novel candidate homologue of human gene *UHRF1BP1L*, it is worthwhile to analyze the *CG34126* mediated model for elucidation of the underlying genetic and cellular basis of the aetiology and pathogenicity of PD.

Homeostasis and Parkinson Disease

Homeostasis is the ability of an organism to keep up a constant internal state during recurrent environmental changes, in such a way that its functioning remains unimpaired (Dobzhansky & Wallace, 1953). Modification evoked by homeostasis facing environmental variation within an organism almost always tend to increase cell proliferation, differentiation, and apoptosis which ultimately lead to the survival and reproduction of the animals (Eijkelenboom & Burgering, 2013). As genetically accessible designs and methodologies available in *Drosophila melanogaster* combine with recent outcomes, it is an ideal model organism to study the pathway of gene regulatory processes and the consequences of their deregulation for PD related neuronal tissue, particularly in

the case of metabolic and proliferative homeostasis (Beckingham *et al.,* 2007). The complex molecular functions of different PD genes regulate various aspects of the cellular homeostasis, such as Parkin's E3 ubiquitin ligase activities on outer mitochondrial membrane stimulate autophagy, *PINK1* activating *parkin* is responsible for mitochondrial dysregulation and neuronal survival or apoptosis, and *DJ-1* acts as an oxidative stress sensor (Drapalo & Jozwiak, 2017; Pickrell & Youle, 2015). Dopaminergic neurons have a vital influence on circadian incapacitation which ultimately leads to circadian system alternation and functional variation in PD (Videnovic & Golombek, 2017). The purpose of this novel *UHRF1BP1L* gene study is to characterize it and determine how its functional cellular adjustments, and correlation with established genes contribute in developing homeostasis occurrences in humans with PD through the *Drosophila* model.

Goals and Objectives

To understand the role of human gene *UHRF1BP1L* in Parkinson Disease, I will investigate the consequences of altering expression of the homologue in *Drosophila melanogaster* to make novel model of Parkinson Disease. In brief, the following goals and objectives are the central focus of this academic work:

1. Explore the conservation in Drosophila of the recently identified PD gene, *UHRF1PB1L* through bioinformatic analysis.

2. Evaluate the effects, if any, upon expression of *CG34126* in the developing *Drosophila melanogaster* eye.

3. Determine if inhibition and overexpression of *CG34126* may influence longevity and/or climbing ability over time.

4. Determine if alteration of *CG34126* expression may influence longevity and/or climbing ability over time in the previously established *park* loss-of-function *Drosophila melanogaster* model of PD.

Methods and Materials

Bioinformatic assessment

A number of bioinformatic tools were applied to understand the potential biological function of the *Drosophila melanogaster* homologue of the human gene *UHRF1BP1L.* The nucleotide sequence of the human PD candidate gene *UHRF1BP1L* (NC_000012.12), the homologueous gene of *Drosophila melanogaster CG34126 (*NT_033779.5) and other species genes were sourced from National Center for Biotechnology Information (NCBI) website (*https://www.ncbi.nlm.nih.gov)* and fly Base website *(https://flybase.org/).* To identify the *Drosophila melanogaster* homologue (NT_033779.5) of human *UHRF1BP1L,* a translated nucleotide data base using protein query search (tBLASTn) was performed using the Basic Local Alignment Search Tool BLAST *(www.ncbi.blast.com).* For multiple sequence alignment, Cluster Omega *(http://www.ebi.ac.uk/Tools/msa/clustalo/)* and for two sequences, Pairwise Sequence Alignment *(https://www.ebi.ac.uk/Tools/psa/),* were applied to demonstrate identity (fully conserved residues) and similiarity (likelihood for sequence homology) of protein sequences. Conserved domains in UHRF1BP1L protein sequences of both vertebrate and invertebrate species were identified using the Conserved Domain Database (CDD) (Derbyshire *et al*., 2015; Geer *et al.,* 2002) tools of NCBI (*https://www.ncbi.nlm.nih.gov/cdd)* and domain identification software Pfam (Mizuno *et al.* 2007) *(https://pfam.xfam.org/).*

Drosophila stocks and derivative lines

The stocks used to direct the overexpression of CG34126, *y[1] w[67c23]; P{w[+mC] y[+mDint2] =EPgy2} EY11029* (designated as *UAS-CG34126*^{EY}) were

obtained from the Bloomington Drosophila Stock Center, Indiana University, Bloomington, USA (stock number BDSC:20245). The stocks utilized to direct the RNA-interference of CG34126, *P{KK108307} VIE-260B* (designated as *UAS-CG34126-RNAi*^{KK}) and *w[1118]; P{GD11162} v26336* (designated as *UAS-CG34126-RNAi*^{GD}) were obtained from the Vienna Drosophila Resource Center, Austria (stock numbers VDRC107307 and VDRC26336, respectively). Detailed information about these stocks are available from http://www.flybase.org. With regards to the *Gal4* bearing transgenic lines, the *dopa decarboxylase* (Ddc)-*Gal4* fly line (BDSC7010) (Li *et al.,* 2000) was provided by Dr. J. Hirsh (University of Virginia). The *tyrosine hydroxylase (TH)-Gal4,* (BDSC:8848) (Friggi *et al*., 2003), g*lass multiple reporter (GMR)-Gal4* (BDSC:1104) (Freeman, 1996), *D42-Gal4* (BDSC:8816) (Yeh *et al.,* 1995), and control line *UAS-lacZ* (BDSC:1776) (Brand *et al.,* 1994) were obtained from the Bloomington Drosophila Stock Centre at Indiana University. Derivative line *Ddc-Gal4/CyO; UAS-park-RNAi/TM3* was generated and tested in the Dr. Brian E Staveley lab using standard homologous recombination methods. See Table 2 for a full list of all genotypes used.

Male progeny was selected to observe *CG34126* gene effect on flies to keep up consistency throughout the whole experiment. Moreover, reproductive stress is significant in females as far as ageing is concern and isolating virgin females could make this experiment much more time-consuming. However, the evaluation of females can certainly be done in future.

Table 2: Genotypes of all stocks used to characterize *CG34126* in this study

Genotypes	Abbreviation	Expression	Balancer	References
Control Line ***w; UAS-lacZ$^{4\text{-}1\text{-}2}$***	*UAS-lacZ*			(Brand *et al*., 1994)
***Gal4* directed expression Lines**				
w;GMR-Gal4^{12}	*GMR-Gal4*	Eye		(Freeman, 1996)
w[*]; P{w[+mW.hs]=GawB}D42	*D42-Gal4*	Motor neurons		(Yeh *et al.,* 1995)
w^{1118}; P{Ddc-Gal4.L}$^{4.3D}$	*Ddc-Gal4*	Dopaminergic and other Neurons		(Li *et al*., 2000)
w*; P{ple-Gal4.F}3	*TH-Gal4*	Dopaminergic Neurons		(Friggi-Grelin *et al*., 2003)
Experimental Lines				
B20245: y [1] w[67c23]; P{w[+mC] y[+mDint2] =EPgy2} EY11029	*UAS-CG34126^{EY}*			(Bellen *et al*., 2011)
w^{1118}; P{GD11162}v26336	*UAS-CG34126-RNAi^{GD}*			(Jansen *et al*., 2017)
P{KK108307}VIE-260B	*UAS-CG34126-RNAi^{KK}*			(Jansen *et al*., 2017)
Derivative Lines ***w; ddc-Gal4/CyO; UAS-park-RNAi/ TM3***	*Ddc-Gal4; UAS-park-RNAi*	Dopaminergic Neurons	CyO; Curly wings (Curly) TM3; Tubby Body	Staveley research group

Media and culture

Stocks of *Drosophila melanogaster* were reared in a standard cornmeal, yeast, molasses and agar medium. The medium used by the research group of Dr. Brian Staveley is composed of 65 g/L cornmeal, 15 g/L nutritional yeast, and 5.5 g/L agar in distilled water which is sterilized by autoclave. During cooling, 50 ml/L fancy grade molasses was added and then, to counter spoilage, 5 ml of 0.1 g/mL methyl-4-hydroxybenzoate paraben in (95% ethanol) and 2.5 mL of propionic acid were added prior to being aliquoted. Prepared medium was poured into plastic vials, refrigerated at 4 °C to 6 °C for solidification up to 3 weeks and equilibrated to room temperature before use. Stocks were raised and moved into new media every 2 or 3 days to avoid crowdedness and increase breeding rate (Merzetti *et al.,* 2016). Male and female flies were crossed to observe phenotypic expression of the target gene and crosses and experiments were kept at 25 °C.

Biometric analysis of *Drosophila* eye

The compound eye of *Drosophila* was taken to examine phenotypic characters such as eye shape, ommatidia, and bristle number. Male flies of each individual cross were collected in groups of up to 20 after eclosion and matured for 2 or 3 days on standard media, followed by freezing at -80 °C. Selected flies were mounted on scanning electron microscope studs placing left eyes upwards, desiccated overnight, and eye images were taken using the FEL Mineral Liberation Analyzer 650F, located at Bruneau centre for innovation and research, and magnification ranges from 550X - 650X. At least 10 micrographs of each cross were analyzed by the National Institute of Health (NIH) ImageJ software (*https://imagej.nih.gov/ij/download.html)* and GraphPad Prism version 8.0.0 was

used for performing biometric assay (Schneider *et al.,* 2012). Comparison of the measured parameters was done by t-test ($p<0.05$).

Lifespan analysis

Vials containing flies were kept at temperature 25 °C for results comparison and up to two hundred adult male flies of each genotype were collected and from them twenty flies were stored in per vial on fresh media. Every 48 hours, flies were checked for viability, media were changed twice in a week or whenever deceased flies were found. Analysis of survival data was carried out using GraphPad Prism version 8.0.0 and different curves are compared using the log-rank (Mental-Cox) test (M'Angale & Staveley, 2016b). Significance was determined at 95%, at a P value less than or equal to 0.05 with Bonferroni correction.

Climbing analysis

Locomotor ability represents the motor control of flies throughout their lifespan. The climbing equipment was a glass tube which is 30 cm in length and 1.5 cm in diameter. It was labelled with a series of five lines, each 2 cm apart (scored 1-5), with a buffer region at the top of the apparatus. Fifty male progenies of each genotype were collected after eclosion and separated into a group of ten flies. Seven days after collection and every week thereafter, flies were assessed for 10 seconds climbing to 10 times per vial of 10 flies. Flies were tranferred from vial to climbing tube carefully without using CO_2. Results were calculated by the following formula:

Climbing index = $\sum$ (nm) / N

where n=number of flies at a given level, m=the score for that level (1-5) and N=total number of flies climbed for that trial. After examining the climbing ability, a regression curve was applied with a 95 % confidence interval to analyze the graphs of 5-climbing index within a given time for each genotype. The slope for each graph represents the rate of decline in climbing ability and the Y-intercept represents the initial climbing ability; both slope and Y-intercept were not constant across all groups, so it was needed to incorporate parameters into statistical analysis to determine the variation in climbing ability. (Merzetti, E. M. & Staveley, 2016; Todd & Staveley, 2004). A comparison of fits demonstrated whether or not curves varied between groups.

Results

Bioinformatics analysis

Identification of the *Drosophila melanogaster* homologue of *UHRF1BP1L*

The amino acid sequence of the *Homo sapiens* UHRF1BP1L protein (NP_055869.1) of 1464 residues was obtained from the NCBI website. A tBLASTn search of the *Drosophila melanogaster* genome was conducted and gene *CG34126* (NP_001260065.1) was identified as the protein sequence most similar to *Homo sapiens* UHRF1BP1L, with 1475 amino acids. These two sequences were aligned using, Pairwise Sequence Alignment and Clustal Omega multiple sequence alignment to identify regions and percentage of similarity. The proteins share 405 identical, 397 highly conserved, and 99 less conserved amino acids; the overall identity and similarity between the human and *Drosophila melanogaster* proteins were 26.5% and 41.5%, respectively (Figure 2). *UHRF1BP1* is the paralogue gene of human *UHRF1BP1L*. The human protein sequence of UHRF1BP1 protein of 1440 amino acids was obtained from NCBI (NP_060224.3) and it was aligned with the *Drosophila melanogaster* CG34126 (NP_001260065.1) amino acids sequence. The proteins share 309 identical, 294 highly conserved, and 192 less conserved amino acids; the overall identity and similarity between the proteins were 26.7% and 42.5%, respectively. The conserved Domain Database (CDD) of NCBI and Pfam were used for the identification of domains Chorein-N, VPS13, ID/GR, FNIII, and coiled-coil (CC) (Figure 2 and 4). Pairwise Sequence Alignment of UHRF1BP1L domains with CG34126 showed Chorein-N (identity-57.2%; similarity-72.2%), VPS13 (identity-37.3%; similarity-55.4%), FNIII (identity-21.2%; similarity- 46.2%), ID/GR (identity-13.3% and similarity-20.1%), and CC (identity-6.8% and similarity-13.6%) (Figure 3).

```
                              Chorein_N

Drosophila    MVSLIKNQLLKHLSIYTKNLSSDKINLSTFRGEGELSNLELDERVLTELLELPSWLRLTS    60
Homo1         MAGIIKKQILKHLSRFTKNLSPDKINLSTLKGEGQLTNLELDEEVLQNVLELPTWLAITR    60
Homo1like     MAGIIKKQILKHLSRFTKNLSPDKINLSTLKGEGELKNLELDEEVLQNMLDLPTWLAINK    60
              *..:**:*:***** :***** *******::***:*.******.** ::*:**:** :.

Drosophila    AWCNHVSFRISWTKLKSVPITLTLDEVRITIETCNPTTRDAGGGSGAGGAGSPTAAASAA    120
Homo1         VYCNRASIRIQWTKLKTHPICLCLDKVEVEMKTCEDPRPPNGQS--------PIA-----    107
Homo1like     VFCNKASIRIPWTKLKTHPICLSLDKVIMEMSTCEEPRSPNGPS--------PIA-----    107
              .:**:.*:** *****: ** * **:* : :.**:        * .        * *
                                                            VPS13
Drosophila    LPQVPQGKYSFIHKVVDGITIVVNTVNVNFVSAAFTASVQMSRIRVESKTPKWANADLRL    180
Homo1         -LASGQSEYGFAEKVVEGMFIIVNSITIKIHSKAFHASFELWQLQGYSVNPNWQQSDLRL    166
Homo1like     -TASGQSEYGFAEKVVEGISVSVNSIVIRIGAKAFNASFELSQLRIYSVNAHWEHGDLRF    166
                   *.:*.* .***:*: : **:: :.: : ** **.:: :::  * . :* :.***:

Drosophila    TRLKDAQKGIILIFKELSWQTVRIEASSTQD----KSLTPLRLLTNHARCRITIRKRLSD    236
Homo1         TRITDPCRGEVLTFKEITWQTLRIEADATDNGDQDPVTTPLRLITNQGRIQIALKRRTKD    226
Homo1like     TRIQDPQRGEVLTFKEINWQMIRIEADATQSSHLEIMCAPVRLITNQSKIRVTLKRRLKD    226
              **: *  :* :* ***:.** :****.:*:.        :*:**:**:.: ::::::* .*

Drosophila    CSLLASRLVLILDDLLWVLTDSQLKAALHFVDSLSGLIKAATHATQKTKAARKMQTLPEY    296
Homo1         CNVISSKLMFLLDDLLWVLTDSQLKAMMKYAESLSEAMEKSAHQRKSLAPEPVQITPPAP    286
Homo1like     CNVIATKLVLILDDLLWVLTDSQLKAMVQYAKSLSEAIEKSTEQRKSMAPEPTQSSTVVA    286
              *.:::::*:::*************** :::..***  :: ::.  :.         :

Drosophila    KAQVEQQQ----NRLSESAHTTNAQRMFNAFDVRETSYHFFSQRIDLHLCDDEGDGRS-S    351
Homo1         SAQQSWAQAFGGSQGNSNSSSSRLSQYFEKFDVKESSYHLLISRLDLHICDDSQSREPGV    346
Homo1like     SAQQVKTT----QTSNAPDVNDAIVKLFNDFDVKETSHHLVISHLDLHICDDIHAKEK-E    341
              .**         .  .    .    : *: ***:*:*:*:. .::***:***       .

Drosophila    YPDLDKGGALQVSVTAFQVDYYPYHLAKSDRSHWAKYKEASVAPALWLKESLNAFREAVL    411
Homo1         SANRLMGGAMQLTFRKMAFDYYPFHWAGDSCKHWVRHCEAMETRGQWAQKLVMEFQSKME    406
Homo1like     SNRRITGGAMQLSFTQLTIDYYPYHKAGDSCNHWMYFSDATKTKNGWANELLHEFECNVE    401
                    ***:*::.  : .****:* * .. .**  . :*  :   * :: :  *.  :

Drosophila    NLSQPNRPA--THAPLERSTPASPIMLSASMLGSQHGAGSFSNGSSTPTAAGLAAGS--G    467
Homo1         KWHEETGLKPPWHLGVD-----------SLFRRKADSLS---SPRKNPLERSPSQG----    448
Homo1like     MLKQAVK--------------------------DHNVG---SPPKSPTHASPQHTQTEK    431
                 :                           .. .   .  ..*   .
```

```
Drosophila    GSAGSGTASMNSQFSQAAQQRSTLENLAKLMSSCVILRIEDFTLYRVTTSGKK-AMPKEF    526
Homo1         --------RQ-PAF--------QPPAWNRLRSSCMVVRVDDLDIHQVSTAGQPSKKPSTL    491
Homo1like     DYPLKGTCRTPSVL--------SQQSKAKLMSSSVVVRLADFNIYQVSTAEQCRSSPKSM    483
                      :             :* **.:::*: *: :::*:*: :    *. :

Drosophila    VSGDKDRYSFPAEMPIIHAEYTYFYYPGDFVFPLPPSKVFVHVNPIQVHFDLSSILWLNS    586
Homo1         LSCSRKLHNLPTQVSAIHIEFTEYYFPDNQELPVPCPNLYIQLNGLTFTMDPVSLLWGNL    551
Homo1like     ICCNKKSLYLPQEMSAVYIEFTEYYYPDGKDFPIPSPNLYSQLNALQFTVDERSILWLNQ    543
              :. .:.   :* ::  :: *:* :*:*..  :*:*  ::: ::* : . .*  *:** *

Drosophila    FGLNLHESLLRTSVGSQSTLHPQQQLPRGSIASNGSNGTQMAAVNVEQEPNLMYMDVKVE    646
Homo1         FCLDLYRSLEQFKAIYK--------LE------------------DSSQKDEHLDIRLD    584
Homo1like     FLLDLKQSLNQFMAVYK--------LN------------------DNSKSDEHVDVRVD    576
              * *:* .** :  .  :        *                  :.. .  ::*::::

Drosophila    AIMPRIVMEAALDAPSQKDRPKTMQIQVSRFALTNIREMGSSRADLAQALHSLQEGSLVF    706
Homo1         AFWLKVSFPLEKRERAELHRPQALVFSASGMIATNTRHAPHCSCSDLQSLFRGFAAAEFF    644
Homo1like     GLMLKFVIPSEVKSECHQDQPRAISIQSSEMIATNTRHCPNCRHSDLEALFQDFKDCDFF    636
              .:  :. :       .. .:*::: :. * :  ** *.   .  .  ::*.     . .*

Drosophila    G---SGFPSKEGDMCIVTDRILSHVAASDVSMMSPVSPTGQQLPRSASTQYLSRYVMWLE    763
Homo1         HSNYDHFPKVPGGFSLLHMLFLHHAFQMDSCLPQP--NT--L-----------PPQRPKA    689
Homo1like     SKTYTSFPKSCDNFNLLHPIFQRHAHEQDTKMHEIYKGN--ITP-----QLNKNTLKTSA    689
                    **.  ..: ::   :  *.   *  : .    .

Drosophila    PRDVWCIKLDPVWVDFLGARSLGPNKSIPFVDAVPITLWLHSGSAQAQLDVG-----KSG    818
Homo1         SWDLWSVHFTQISLDFEGTEN-FKGHTLNFVAPFPLSIWACLPLRWQQAQARKLLLASEG    748
Homo1like     ATDVWAVYFSQFWIDYEGMKS-GKGRPISFVDSFPLSIWICQPTRYAESQKEPQTCNQV-    747
                *:*.: :  . :*: * ..   .: : **  .*:::*         : :        .
```

NLS

ID/GR

```
Drosophila    TAGSMESMGMP-------------PLPTLPPLQPCNPFLSDEDVRLAG----VASGASP    860
Homo1         RLKPSASFGSPVQSEALAPDSMSHPRSKTEHDLKSLSGLTEVMEILKEGS---SGMDNKG    805
Homo1like     SLNTSQSESSD-----LA------GRLKRKKLLKEYY--STESEPLTNGGQKPSSSDTFF    794
                  * .                       *:          :    *      . .

Drosophila    PAPAPDRTADVHAIAHISNLVSLQIDHYQLLFLLRLAEELNEMSTFLNLDAERILQKQNE    920
Homo1         PLTELEDVADVHMLVHSPAHVRVRLDHYQYLALLRLKEVLQRLQEQLTKDTESMTGSPLQ    865
Homo1like     RFSPSSSEADIHLLVHVHKHVSMQINHYQYLLLLFLHESLILLSENLRKDVEAVTGSPAS    854
                  .  **:* :.*    * ::::*** * ** * * *  :.  *  *.* :  .  .
```

```
Drosophila      QKSIIFGCVVPQIEVTLVMPSPTPGGNITWP-------TPPPLDQLKSNTFGSVE-----    968
Homo1           NQTACIGVLFPSAEVALLMHPAPGAVDADSAGSDSTSLVDSELSPSEDRELKSDASSDQG    925
Homo1like       QTSICIGILLRSAELALLLHPVDQANTLKSPVSESVSPVVPDYLPTENGDFLSSKRKQIS    914
                : :  :* :. . *::*::     .             .       :.  : *

Drosophila      T--PSPVTNEPPFDNGIHISNPNTHGYNV-QIQ-STPTMASSTASQGSRPDTGISTQSQS    1024
Homo1           PASPEKVLEESSIEN-QDVSQERPHSN--GELQDSGPLAQQ-------LAGKGHEAVESL    975
Homo1like       R----DINRIRSVTV-NHMSDNRSMSVDLSHIPLKDPLLFKS-AS-DTNLQKGISFMDYL    967
                      : .   .    .:*: .  .     .:  . *   .            .* .  .

Drosophila      QTQSISSASKS------AKSAAARSGAT--DTVPSLTKEINSGLLSMKKG--FSSFMT--    1072
Homo1           QAKKLSRTQASSSPAA-LKPPAG---RETA------VN-GQGELIPLKNI----------    1014
Homo1like       SDKHLGKISEDESSGLVYKSGSGEIGSETSDKKDSFYT-DSSSILNYREDSNILSFDSDG    1026
                . : :.  . .       *  :.               .   .. ::  ::

Drosophila      ---SIDSAIKSGTPNDDASDTFSIQSDISSDSDN---FANVLGDDKTMDCMDVMFRLNPF    1126
Homo1           -EGELSSAIHMT--KDATKEALHATMDLTKEAVSLTKDAFSLGRDRMTSTMHKMLSLPPA    1071
Homo1like       NQNILSSTLTSK--GNETIESIFKAEDLLPEAASLSEN-LDISKE----------ETPPV    1073
                    :.*::      : : :::    *:  :: .       :. :              *

Drosophila      TTDN------------------------MKA-------SPVEVASEVYEEQ-------    1146
Homo1           KEPMAK----TDEGVAAPVSGGAARLRFFSMKRTVSQQSFDGVSLDSSGPEDRISVDSDG    1127
Homo1like       RTLKSQSSLSGKPKERCPPNLAPLCVSYKNMKRSSSQMSLDTISLDSMILEEQL-LESDG    1132
                                           **         . :.: *   *::

Drosophila      ---------------------------------P--SSYKT-NMSSPSEPSEGSTWRRR    1169
Homo1           SDSFVMLLESESGPESVPPGSLSNVSDNAGVQGSPLVNNYGQGSPAANSSVSPSGEDLIF    1187
Homo1like       SDSHMFLEKGNKKNSTT-----NYRGTAESVNAGANLQNYGETSPDAISTNSEGAQENHD    1187
                                                      ..*   .  : *  * ..

Drosophila      DLVSMATFRLTTVELIRQQEGPKSSVRLQVAAVSCDECGAIPWDELQIARQANKTKFGAR    1229
Homo1           HPVSVLVLKVNEVSFGIEVRGEDLTVALQAEELTLQQLGTVGLWQFLHGQCP------GT    1241
Homo1like       DLMSVVVFKITGVNGEIDIRGEDTEICLQVNQVTPDQLGNISLRHYLCNRPV------GS    1241
                . :*: .:::. *.    : .* .  : **.  :: :: * :   .    :        .

Drosophila      CKAWNLAPYNPEAPPCIRMRLEETLNMPKEIEGIIDRKRIQSWITHHAEIRVKDINMDLS    1289
Homo1           CFQESSTLKTGHIRPAVGLRFEVGPGAA--VHSPLAS------QNGFLHLLLHGCDLELL    1293
Homo1like       ---DQKAVIHSKSSPEISLRFESGPGAV--IHSLLAE------KNGFLQCHIENFSTEFL    1290
                    . :     .  * : :*:*  .     :.. :          . . .  :.. . ::
```

FNII

```
Drosophila      MSTVIGLGDLAEDEVISPPMPLTVNLENVRINLLEDRPPVNITSPGPIPINLCIGRMRLE    1349
Homo1           TSVLSGLGPFLEDEEIPVVVPMQIELLNSSITLKDDIPPIYPTSPGPIPITLAMEHVVLK    1353
Homo1like       TSSLMNIQHFLEDETVATVMPMKIQVSNTKINLKDDSPRSSTVSLEPAPVTVHIDHLVVE    1350
                 * : .:  : *** :   :*: ::: *  *.* :* *    .*  * *:.: : :: ::

Drosophila      RDQSGLLNIQPIDTNMSDAQHQ--A-LGSALFGAPRERDRELLSMQLVMQQMKLDND-QL    1405
Homo1           RSDDGVFHIGAAAQDKPSAEVLKSE----------K-RQ--P----PKEQVFLVPTGEVF    1396
Homo1like       RSDDGSFHIRDSHMLNTGNDLKENVKSDSVLLTSGK-YD--LKKQRSVTQATQTSPGVPW    1407
                *.:.* ::*        . :               :  :          *      .

Drosophila      RRQLVD-SKVNTDNYRHKTKQEADVLRSYLKAAQDDISILLEEKKALLDTIRSLQVQLTS    1464
                                                         CC
Homo1           EQQV----------------KELPILQKELIETKQALANANQDKEKLLQEIRKYNPFFEL    1440
Homo1like       PSQSANFPEFSFDFTREQLMEENESLKQELAKAKMALAEAHLEKDALLHHIKKMTVE---    1464
                  *                 :*   *:. *  ::  ::    :*. **. *:.

Drosophila      SNMSRKSDGNR    1475
Homo1           -----------    1440
Homo1like       -----------    1464
```

Figure 2. Alignment of protein encoded by *Drosophila melanogaster* CG34126 with the two human UHRF1BP1 and UHRF1BP1L protein.

Clustal Omega multiple sequence alignment of human UHRF1-binding protein1 (UHRF1BP1) (Homo1 is *Homo sapiens* NP_060224.3) and human UHRF1-binding protein1-like protein (UHRF1BP1L) (Homo1like is *Homo sapiens* NP_055869.1) with the *Drosophila melanogaster* CG34126 protein (Drosophila is *Drosophila melanogaster* NP_001260065.1) showing the highlighted domain Chorein-N (black), VPS13(yellow), ID/GR (light blue), FNIII (oranke), CC (light pink), and NLS (purple). The domains were identified using CD-search tool of NCBI Conserved Domain Database Search (CDD) and Pfam. "*" (Asterisk) indicates the residues that are identical, ":" (Colon) indicates the strongly similar, "." (Period) indicates the weakly similar. Colors show the chemical nature of amino acids. Red is small hydrophobic (including aromatic Y), Blue is acidic, Magenta is basic, and Green is basic with hydroxyl or sulfhydryl or amine groups. Grey is unusual amino acids.

```
Homo1like              1 IIKKQILKHLSRFTKNLSPDKINLSTLKGEGELKNLELDEEVLQNMLDLP     50
                         :||.|:|||||.:|||||.|||||||.:|||||.||||||.||..:|:||
Drosophila             1 LIKNQLLKHLSIYTKNLSSDKINLSTFRGEGELSNLELDERVLTELLELP     50

Homo1like             51 TWLAINKVFCNKASIRIPWTKLKTHPICLSLDKVIMEMSTCEEPR--     95
                         :||.:...:||..|.||.|||||:.||.|:||:|.:.:.||....
Drosophila            51 SWLRLTSAWCNHVSFRISWTKLKSVPITLTLDEVRITIETCNPTTRD     97
```

Chorein-N

```
Homo1like              1 --------------RIGAKAFNASFELSQLRIYSVNAHWEHGDLRFTRIQ     36
                                       ...:.||.||.::|::|:.|....|.:.|||.||::
Drosophila             1 VVDGITIVVNTVNVNFVSAAFTASVQMSRIRVESKTPKWANADLRLTRLK     50

Homo1like             37 DPQRGEVLTFKEINWQMIRIEADATQSSHLEIMCAPVRLITNQSKIRVTL     86
                         |.|:|.:|.|||::||.:||||.:||...|    .|:||:||.::.|:|:
Drosophila            51 DAQKGIILIFKELSWQTVRIEASSTQDKSL----TPLRLLTNHARCRITI     96

Homo1like             87 KRRLKDCNVIATKLVLILDDLLWVLTDSQLKAMVQYAKSLSEAIEKSTE-    135
                         ::||.||:::|::|||||||||||||||||||||.:.:..|||..|:.:|.
Drosophila            97 RKRLSDCSLLASRLVLILDDLLWVLTDSQLKAALHFVDSLSGLIKAATHA    146

Homo1like            136 QRKSMAPEPTQSSTVVASAQQVKTTQ--------TSNAPDVNDAIVKLFN    177
                         .:|:.|....|  |:.....||:..|        |:||.       ::||
Drosophila           147 TQKTKAARKMQ--TLPEYKAQVEQQQNRLSESAHTTNAQ-------RMFN    187

Homo1like            178 DFDVKETSHHLVISHLDLHICDDIHAKEKESNRRIT------GGAMQLSF    221
                         .|||:|||:|.....:|||:|||      |.:.|.:      |||:|:|.
Drosophila           188 AFDVRETSYHFFSQRIDLHLCDD------EGDGRSSYPDLDKGGALQVSV    231

Homo1like            222 TQLTIDYYPYH    232
                         |...:|||
Drosophila           232 TAFQVDYY---    239
```

VPS13

```
Homo1like              1 --IHSLLAEKNGFLQCHIENFSTEFLTSSLMNIQHFLEDETVATVMPMKI     48
                           |.|.:..   ..:..:::.:.:...|:::.:....|||.::..||:.:
Drosophila             1 KRIQSWITH---HAEIRVKDINMDLSMSTVIGLGDLAEDEVISPPMPLTV     47

Homo1like             49 QVSNTKINLKDDSPRSSTVSLEPAPVTVHIDHLVVERSDDGSFHIR--DS     96
                         .:.|.:|||.:|.|..:..|..|.|:.:.|..:.:||...|..:|:  |:
Drosophila            48 NLENVRINLLEDRPPVNITSPGPIPINLCIGRMRLERDQSGLLNIQPIDT     97

Homo1like             97 HMLNTGNDLK-------    106
                         :|    :|.:
Drosophila            98 NM----SDAQHQALGSA    110
```

FNIII

```
Homo1like      1 --------------------------------------------------    0

Drosophila     1 SPTGQQLPRSASTQYLSRYVMWLEPRDVWCIKLDPVWVDFLGARSLGPNK   50

Homo1like      1 -----------------------------------------------PQT    3
                                                                .|.
Drosophila    51 SIPFVDAVPITLWLHSGSAQAQLDVGKSGTAGSMESMGMPPLPTLPPLQP  100

Homo1like      4 CNQVSLNTSQSESSDLAGRLKRKKLLKEYYSTESEPLTNGGQKPSSSDTF   53
                 ||..    ...|...|||                  :.:|...|:.
Drosophila   101 CNPF----LSDEDVRLAG------------------VASGASPPAP----  124

Homo1like     54 FRFSPSSSEADIHLLVHVHKHVSMQINHYQYLLLLFLHESLILLSENLRK  103
                    :|..: ||:|.:.|:...||:||:|||.|.||.|.|.|..:|..|..
Drosophila   125 ---APDRT-ADVHAIAHISNLVSLQIDHYQLLFLLRLAEELNEMSTFLNL  170

Homo1like    104 DVEAVTGSPASQTSICIGILLRSAELALLL--------------HPVDQ-  138
                 |.|.:......|.||..|.::...|:.|::              .|:||
Drosophila   171 DAERILQKQNEQKSIIFGCVVPQIEVTLVMPSPTPGGNITWPTPPPLDQL  220

Homo1like    139 -ANTLKSPVSESVSPVVPDYLPTENG-------------------DFLSS  168
                  :||..|  .|:.|||..: .|.:||                   ...||
Drosophila   221 KSNTFGS--VETPSPVTNE-PPFDNGIHISNPNTHGYNVQIQSTPTMASS  267

Homo1like    169 KRKQISRDINRIRSVTVNHMSDNRSMSVDLSHIPLKDPLLFKSASDT---  215
                 ...|.||.     ...::..|.:::.|:..:....|.......|:||
Drosophila   268 TASQGSRP-----DTGISTQSQSQTQSISSASKSAKSAAARSGATDTVPS  312

Homo1like    216 ----------NLQKGI-SFMDYLSDKHLGKISEDESSGLVYKSGSGEIGS  254
                           :::||. |||
Drosophila   313 LTKEINSGLLSMKKGFSSFM------------------------------  332

Homo1like    255 ETSDKKDSFYTDSSSILNYREDSNILSFDSDGNQNILSSTLTSKGNETIE  304

Drosophila   333 --------------------------------------------------  332

Homo1like    305 SIFKAEDLLPEAASLSENLDISKEE    329

Drosophila   333 -------------------------    332
```

ID/GR

```
Homo1like      1 -----------------REQLMEENESLKQELAKAKMALAEAHL     27
                                  ...|:||.::
Drosophila     1 KQEADVLRSYLKAAQDDISILLEEKKA-----------------     27
```

Coiled-Coil

Figure 3. Pairwise Sequence Alignment of UHRF1BP1L and CG34126 domains.

Alignment of UHRF1BP1L domains with CG34126 showed Chorein-N (identity-57.2%; similarity-72.2%), VPS13 (identity-37.3%; similarity-55.4%), FNIII (identity-21.2%; similarity- 46.2%), ID/GR (identity-13.3% and similarity-20.1%), and CC (identity-6.8% and similarity-13.6%).

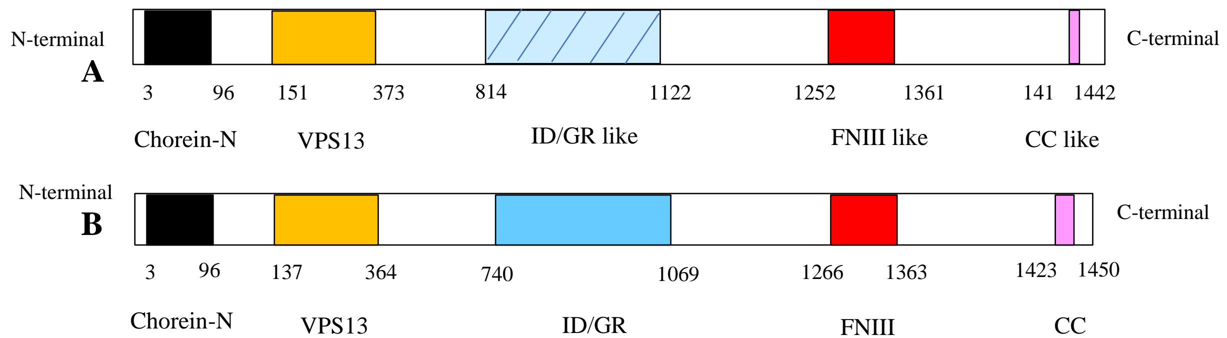

Figure 4. (A) *Drosophila melanogaster* CG34126 protein (1475 amino acid residues) and (B) *Homo Sapiens* UHRF1BP1L protein (1464 amino acid residues) with conserved domains.

Highlighted domains are Chorein-N (black), VPS13 (Yellow), ID/GR (light blue), FNIII (oranke), and CC (pink)

UHRF1BP1L and CG34126 are conserved between vertebrates and invertebrates

A BLASTn search of NCBI identified potentially homologous versions of vertebrate and invertebrate *UHRF1BP1L*-related-protein, including *Homo sapiens* UHRF1-binding protein 1-like protein (NP_055869.1), Zebrafish uhrf1bp1l *Danio rerio* (NP_001093475.1), house mouse *Mus musculus* (NP_083442.2), frog uhrf1bp1l *Xenopus laevis* (NP_001084948.1), honey bee (*Apis mellifera* (XP_006559910.2), silk moth (*Bombys mori* XP_012551608.1 and Dmel/CG34126 *Drosophila melanogaster* (NP_001260065.1); were aligned by Clustal Omega multiple sequence alignment to identify amino acids similarity. The multiple sequence alignment of vertebrate and invertebrate *UHRF1BP1L* proteins was performed using the CD-search tool of NCBI Conserved Domain Database Search (CDD) and Pfam for identification of conserved and functional domains; the result showed that Chorein-N and VPS13L domains were all highly conserved among the different proteins (Figure 5).

```
                                          Chorein_N

Danio        MAGLIKKQILKHLSRFAKNLSPDKINLSTLKGEGQLTNLELDEEVLQNMLDLPTWLAINK   60
Xenopus      MAGLIKKQILKHLSRFTKNLSPDKINLSTLKGEGQLTNLELDEEVLQNMLDLPTWLAINK   60
Homo         MAGIIKKQILKHLSRFTKNLSPDKINLSTLKGEGELKNLELDEEVLQNMLDLPTWLAINK   60
Mus          MAGIIKKQILKHLSRFTKNLSPDKINLSTLKGEGELKNLELDEEVLQNMLDLPTWLAISK   60
Bombys       MVTIIKNQLLKHLSRFTKNLSPEQISLSALRGSGELQDLTLDEDLLTDLLELPGWLRLTS   60
Drosophila   MVSLIKNQLLKHLSIYTKNLSSDKINLSTFRGEGELSNLELDERVLTELLELPSWLRLTS   60
Apis         MVSLIKKQLLKHLSRFTKNLSADKINLSTFKGEGELTNLELDEIVLTDLLELPSWLRLTN   60
             *. :**:*:***** ::**** ::*.**:::*.*:* :* *** :* ::*:** ** :..

Danio        VFCNKAAIRIPWTKLKTHPISLSLDKVVMEMSTCDEPRPPNGPSPIA-------------   107
Xenopus      VFCNKAAIRIPWTKLKTHPISLSLDKVIMEMSTCEEPRSCNGPSPLV-------------   107
Homo         VFCNKASIRIPWTKLKTHPICLSLDKVIMEMSTCEEPRSPNGPSPIA-------------   107
Mus          VFCNKASIRIPWTKLKTQPICLSLDKVIMEMSTCEEPRAPNGPSPIA-------------   107
Bombys       AKCNRASFRIQWTKLKTVPIVLNLDEVHIALEVCSEPRVMNPSAAG--------------   106
Drosophila   AWCNHVSFRISWTKLKSVPITLTLDEVRITIETCNPTTRDAGGGSGAGGAGSPTAAASAA   120
Apis         AWCNKVSFRIQWTKLRSVPIFLSLDEVHIEVETCEDLRDLS-SSQGLS------------   107
             . **:.::** ****:: ** *.**:* : :..*.          .

                                                       VPS13

Danio        -TASGQSEYGFAEKVVEGISLSVNSIVIRISAKAFNASFELSQLQVYSVNTSWTTGDLRY   166
Xenopus      -TASGQSEYGFAEKVVEGISLSVNSIIIRIRAKAFNASFELSQLRIYSVNPSWQHGDLRF   166
Homo         -TASGQSEYGFAEKVVEGISVSVNSIVIRIGAKAFNASFELSQLRIYSVNAHWEHGDLRF   166
Mus          -TASGQSEYGFAEKVVEGITVSVNSIVIRIGAKAFNASFELSQLRIYSVNAQWEHGDLRF   166
Bombys       -ALPVPGKYSYIHKVIDGISVAVNHVQIDFNCDAFTSSVQISRVTVESRTPDGRKGDLRL   165
Drosophila   LPQVPQGKYSFIHKVVDGITIVVNTVNVNFVSAAFTASVQMSRIRVESKTPKWANADLRL   180
Apis         -SYTGPAKYSFIHKVIDGITVTVNTVSVTFKSPAFIASVQMNRIIVESKSATWQRCDLRT   166
                 .:*.: .**::**:: ** : : : . ** :*.::.:: : * .      ***

Danio        TRILDPTRGELLTFKEVSWQMIRIEADAIQNTEHEVVSAPIRLITNQSKIRVTLKRRMKD   226
Xenopus      TRIQDPQRGEVLTFKEINWQMIRIEADAIQSCDHEIMSAPVRLITNQSKIRITLKRRLKD   226
Homo         TRIQDPQRGEVLTFKEINWQMIRIEADATQSSHLEIMCAPVRLITNQSKIRVTLKRRLKD   226
Mus          TRIQDPQRGEVLTFKEINWQMIRIEADATQSSHLEIMCAPVRLITNQSKIRVTLKRRLKD   226
Bombys       TRIKCTDTGQLLIFKELEWQSARIEAKAHGAASA--NLPPLRLLLGNTHCRIVIKKRLSD   223
Drosophila   TRLKDAQKGIILIFKELSWQTVRIEASST--QDK--SLTPLRLLTNHARCRITIRKRLSD   236
Apis         TRVKDPDRGQLLIFKELEWQTVRIEAQST--KDK--NLTPLRLLTNQARCRITIKKRISD   222
             **:     * :* ***:.**  ****.:         *:**: .::: *:.:::*:.*
```

```
Danio         CNVIASKLTLILDDLLWVLTDSQLKAMVQYAKSLSESMEKSASQRKSMAPDTTQVTPAPP   286
Xenopus       CNVVASKLIFMLDDLLWVLTDSQLKAMVQYAKSLSEAIEKSTEQRKSMASETTQSPTPPV   286
Homo          CNVIATKLVLILDDLLWVLTDSQLKAMVQYAKSLSEAIEKSTEQRKSMAPEPTQSSTVVA   286
Mus           CNVIATKLVLILDDLLWVLTDSQLKAMVQYAKSLSEAIEKSTEQRKSMAPEPTQSSTVTS   286
Bombys        CAVIASRLAICPEPVAWALTDGQLRAALACAAALAQPVRRATAAATRAKALRKIEEPRE-   282
Drosophila    CSLLASRLVLILDDLLWVLTDSQLKAALHFVDSLSGLIKAATHATQKTKAARKMQTLPEY   296
Apis          CFVMGSRLILILDDLLWVLTDSQLKAALHFIDSLGGLIEKATILERKTKAARKLEVLPEY   282
              * ::.::* :  : : *.***.**:* :    :*.  :. ::          .

Danio         TAQQMRTQQASAAADQTASMARLFTAYDVRETSHHLQITHLDLHICDDTNAKDRGINKRL   346
Xenopus       SSQQVKNPQTSTTPEQNNAILKLFRDFDVKETSYHLVISHLDLHICDDIHSKEKAFVRRV   346
Homo          SAQQVKTTQTSNAPDVNDAIVKLFNDFDVKETSHHLVISHLDLHICDDIHAKEKESNRRI   346
Mus           SAQHVKTPQAANAPDLSDAIVKLFNDFDVKETSHHLVISHLDLHICDDIHAKEKESNRRV   346
Bombys        --QI--QSRPSGAAGERDILARVFAKHDVRETSYHLLAPRIDLHLCDDPGLGRSEKPSLS   338
Drosophila    KAQVEQQQNRLSESAHTTNAQRMFNAFDVRETSYHFFSQRIDLHLCDDEGDGRSSYPDLD   356
Apis          QAQISQQSRTK--NQYNTAISKIFTRYDVVETSYHFLCQRIDLHLCDDAGNGRSSHPDLK   340
                *     .            ::*  .** ***:*:   ::***:***

Danio         DGGAMQLSFSSISVDYYPFHKAGEGCLHWMHYGEATKSRETWARSLLDEFKSNVDMLKNA   406
Xenopus       TGGAMQLSFSQLTVDYYPYHREGDGCSHWMHYGDATKTRCSWAQELLHEFNSNIEMLRQA   406
Homo          TGGAMQLSFTQLTIDYYPYHKAGDSCNHWMYFSDATKTKNGWANELLHEFECNVEMLKQA   406
Mus           SGGAMQLSFTQLTIDYYPYHKAGDSCSHWMYFSDATKTKNGWANELLHEFECNVEMLKQA   406
Bombys        KGGALQVTLISMQADLFPYHKASNDRRHWKGYRESATPHSQWLSQALSSFCTNLLETLDP   398
Drosophila    KGGALQVSVTAFQVDYYPYHLAKSDRSHWAKYKEASVAPALWLKESLNAFREAVLNLSQP   416
Apis          DGGALQISLVSFQIDYYPYHLAMSDRKHWAKYKENATPHSQWLQQSLSSFRSQFMDLIDS   400
               ***:*::.  :  * :*:*    ..  **  : : :     *  . *  *   .    :

Danio         VSGQSQGSPQHG----KIS--T--------------S------------------SS   425
Xenopus       VKDHNPSSPIRT----VPN--ASQQYGQTGFDQNVK--------------------RS   438
Homo          VKDHNVGSPPKS----PTH--ASPQHTQTEKDYPLKGT------------------CR   440
Mus           MKDRNLGSPPKS----PTH--ASPQHTQTEKDSTLKGT------------------PK   440
Bombys        RPLSTNNKNQVA----------------RGEETASNGTSQKPAAEAPQRS----ASSVPP   438
Drosophila    NRPATHAPLERSTPASPIMLSASMLGSQHGAGSFSNGSSTPTAAGLAAGSGGSAGSGTAS   476
Apis          GRTQ-HSPLIRSQGNVTV-------NNTKGIGENLEKNN--QAQNVNAIT------HEQK   444
```

```
Danio          ---TSFS-----PPTPPRTQLMSSSIVLRMADFSIYQVSSADQPRS--------SPQTMI   469
Xenopus        ---SPTA-----FGEPPKSNPLSSSFVVRLADFNIFQVSTADQCRS--------SPKTMI   482
Homo           ---TPSV-----LSQQSKAKLMSSSVVVRLADFNIYQVSTAEQCRS--------SPKSMI   484
Mus            ---TPSV-----LPQPSKAKLMSSSVVVRLADFNIYQVSTAEQCRS--------SPKSMI   484
Bombys         ATTTSQPSPTRTRILQRLGKLMTTCLVLRIDNFTVYKVSTGSKTYEALRP----------   488
Drosophila     MNSQFSQAAQQRSTLENLAKLMSSCVILRIEDFTLYRVTTSGKKAMPKEFV---------   527
Apis           KSQHPSGNPVKNYILEQLAKLMTTCIIIRIDDFTLYKVTTTSRNPIPKEFVTAQTRKKHI   504
                                    : :::..::*: :*.:::*::   :

Danio          SCNKKSLYLPQEMPAIHAEFTEYYFPDGKDYPVPCPNLYVQLNALQLVLDSRSLVWLNLF   529
Xenopus        SCNKKSLYLPQEMSAIHIEFTEYYFPDGKNFPIPSPNLYVQLNALQFTLDEKSVLWLNQF   542
Homo           CCNKKSLYLPQEMSAVYIEFTEYYYPDGKDFPIPSPNLYSQLNALQFTVDERSILWLNQF   544
Mus            SCNKKSLYLPQEMSAIYIEFTEYYYPDGKDFPIPSPNLYSQLNALQFTVDERSILWLNQF   544
Bombys         LVNAEKATLPGDAGLLHAELTFFYYPGDDCFPVPAPKLYVQLSPVRVSLDVNSAVWLGAF   548
Drosophila     SGDKDRYSFPAEMPIIHAEYTYFYYPGDFVFPLPPSKVFVHVNPIQVHFDLSSILWLNSF   587
Apis           SGDRDKFCLPEDVTIVHAEFTYYYYPGDITFPLPPPKFYVQLNPIQVNFDVSSCLWFNSF   564
                 : .   :* :   :: * * :*:*..  :*:*  :.: ::. ::. .*  * :*:. *

Danio          ALDLRQSLEQFMDLY---------------------------KLNDSQKPEEHVDIKVDG   562
Xenopus        VLDLRQSLDQFVAMY---------------------------KLSDNSKSDEHVDIRVDG   575
Homo           LLDLKQSLNQFMAVY---------------------------KLNDNSKSDEHVDVRVDG   577
Mus            LLDLKQSLNQFMAVY---------------------------KLNDSSKSDEHVDIRVDG   577
Bombys         LPHVAGALAPPRRGA---------------------------ADPDTPPPAPYMDVRMEA   581
Drosophila     GLNLHESLLRTSVGSQSTLHPQQQLPRGSIASNGSNGTQMAAVNVEQEPNLMYMDVKVEA   647
Apis           ALNLYYSLMNKDKQT-----------------------------TYTSTTLMYFDVKIEA   595
                 .:  :*                                          :.*::::.

Danio          LMLKLVIPADHE--KTQPDQPRSVSVQTSEMVATNTRHSSAC----SRSSLEALLQAFQE   616
Xenopus        LMLKFIIPSQKK-QDCHPDQPAGISIQTSEMIGSNTRQTANC----RRSDLEAIFQDFKD   630
Homo           LMLKFVIPSEVK-SECHQDQPRAISIQSSEMIATNTRHCPNC----RHSDLEALFQDFKD   632
Mus            LMLKFVIPSEVK-AGCHQDQPHTVSIQSSEMIATNTRHCPNC----RHSDLEALCQDFKE   632
Bombys         IMPKIVLEAGSEHVSQQRDRPKELHVCTARAMLTNVRESPRANTAGTRADLAAILSALRR   641
Drosophila     IMPRIVMEAALD-APSQKDRPKTMQIQVSRFALTNIREM-GS----SRADLAQALHSLQE   701
Apis           ILPRIVFESPQD-YPNQKDRPKSLHIQTSRASITNVRCTERS----SRTDLAQCVNAFQM   650
               :: :::: :  .     : *:*  : :  :.   :* *     .     ::.*     ::
```

```
Danio           QPFFTSLSSTFPRAPSSFSVLHSVFQRHAHEQDTRVHDVY--RGLAPPS-----LSTNAL   669
Xenopus         YDFYSKNFTSFPRSHDHFDILHPIFQRHAFEQDTKMHDIY--KGLVAPT-----LDTNAL   683
Homo            CDFFSKTYTSFPKSCDNFNLLHPIFQRHAHEQDTKMHEIY--KGNITPQ-----LNKNTL   685
Mus             CDFFSKTYTRFPKSCDSFNLLHPIFQRHAHEQDTKMHEVY--KGNIIPK-----LNKNTL   685
Bombys          AT-PP--RGEFPASTEDLDPIHEQFVLHADNLDDV---------------DRGTAISPEL   683
Drosophila      GSLVF--GSGFPSKEGDMCIVTDRILSHVAASDVSMMSPVSPTGQQLPRSASTQYLSRYV   759
Apis            GQMFF--STEFPNRSNDFHVLTDKFLAHCAGTDNIRYPPPNFSSNS--VNELIRQLHREL   706
                          **     :  :   :  *    *                          :

Danio           KTPAATDLWAMHFSQFWVDYEGSRS-GRGRPTPCVDSFPLTLWVCHPARYTQHLERLRVG   728
Xenopus         STSAARDIWAIHFTQFWVDYEGIKS-GKGRPVVFIDSFPFSIWICQPRRFLQSQKRTLCD   742
Homo            KTSAATDVWAVYFSQFWIDYEGMKS-GKGRPISFVDSFPLSIWICQPTRYAESQKEPQTC   744
Mus             KTSAATDVWAVYFSQFWIDYEGMKS-GKGRPVSFVDAFPLSIWICQPTRYAELQKEFQTC   744
Bombys          LWRENRSVWCARVEPLWADFCGARATN-YKPAPLLDATPLTAWLCFNDDLS---------   733
Drosophila      MWLEPRDVWCIKLDPVWVDFLGARSLGPNKSIPFVDAVPITLWLHSGSAQAQLDVGKSGT   819
Apis            LWTEAKDVWCCNLEPVWGDFLGARAVGQNRPVPFLDAFPLTLWCYMTMNSLDKD------   760
                      .:*.  .  .* *: * :: .  :    :*: *:: *

Danio           SGL---LPRSESAEMANRLQRKKLLKEYYSTNASPSDTPSNGLHKPQSLDGLFSDSSSSL   785
Xenopus         GDPLQSISKSESTDFVGRLQRKKLLKQYYSTELGGSN---TPLQKSQSLDSSL-----AN   794
Homo            NQVSLNTSQSESSDLAGRLKRKKLLKEYYSTESEPLT---NGGQKPSSSDTFF-----RF   796
Mus             DQVTLNTSQSESSDLAGRMKRKKLLKEYYSTESEPLT---NGGQRPS-SDTFL-----RF   795
Bombys          ------------------------------------------------------------   733
Drosophila      ---------------AGSMESMGMPPL----PTLPPLQPCNPFLSDEDVRLAGVASGASP   860
Apis            ------------------------------------------------------------   760

Danio           PLACSANEVDVQVLVHVQKHLSAQVSHGQYVFLLRLQHAVKTLQRTIQQDLEQYGSKRLG   845
Xenopus         PPTCKQTDADIHVLAHVQKHVSLQINHYQYIFLLLLQESIKQILENVKKDVEEVTG---K   851
Homo            --SPSSSEADIHLLVHVHKHVSMQINHYQYLLLLFLHESLILLSENLRKDVEAVTG---S   851
Mus             --SSSSSDADVHVLVRVHKHVSMQINHYQYLLLLFIHESLVLLSDTLRRDVEAVIG---S   850
Bombys          ---------RIWVVGRTCGLAGLQVNHYQLLFLLRQLERVSEMMAWLAHDASRQ-----P   779
Drosophila      PAPAPDRTADVHAIAHISNLVSLQIDHYQLLFLLRLAEELNEMSTFLNLDAERILQ---K   917
Apis            -SSEKKSTGDIHGLAYISNLVSVQINHYQYLFLLRLSEVLSEMATYLTIDSNKILK---V   816
                          :  :       . *:.* * ::**   . :  :   :  *  .
```

```
Danio         PTQPFSACVGVLMKSAEVALLLKPIPQPDLSTSPLNSDVSPSESRATLEAGSEVSEGHER   905
Xenopus       PDDENKISVGLLLKSADVSLLLLPLPEDN-SKSPLPCEGSPVTDHKLPSPSEGVTG----   906
Homo          PASQTSICIGILLRSAELALLLHPVDQANTLKSPVSESVSPVVPDYLPTENGDFLS----   907
Mus           PASQTSVCVGILLRSAELALLLHPVNPTSALRSPASESGSPLLPDFLPAENGGFLS----   906
Bombys        DAEEGSIVVGLVVPAVELTFVLPSNCPGQESSRD-LDSVPLDSSSLQ----DLKMGSEAT   834
Drosophila    QNEQKSIIFGCVVPQIEVTLVMPSPTPGGNITWPTPPP----LDQLK----SNTFGSVET   969
Apis          D-SGSSLVIGALIPQVEVTFVMPSHTPGKENSGGDLESVMPDSSSIA----DDIAGSS-I   870
                .  .  .* ::   ::::::

Danio         PSAAGEG---TRNVDQLLCADRN--------------SIDTSPLLPSSSSA--LRKTSMD   946
Xenopus       -DLIIN-----NNVDLI-----NKAIFESDLKGDPQKIVATDPLLSKSSSDTELLKRSPP   955
Homo          -SKRKQI---SRDINRIRSVTVNHMSDNRSMSVDLSHIPLKDPLLFKSASDTNLQKG-IS   962
Mus           -SKRKQG---GSGIHRIRNATLNHMSDNRSMSVDLSHAPLKDPLLFKSASDTNLQKG-TS   961
Bombys        MAPSIQD--R--------------------------------------------------   842
Drosophila    PSPVTNEPPFDNGI------HI----SNPN-----THG-------------YNVQIQSTP   1001
Apis          --PWQSTERIESNVKKI---NINNDIITPQ-----SDV--------SSMLSMDFMHSTNP   912
                   .

Danio         --ERSSVASGGRVPSEEKKDLREGSPDGDTSRS--EAKGQQNETSQ--------------   988
Xenopus       YLDSTADKDLLEAEL------AF----GQQID-----RNNLKEDPGANID---------I   991
Homo          FMDYLSDKHLGKISEDESSGLVYKSGSGEIGSETSDKKDSFYTDSSSILNYREDSNILSF   1022
Mus           FLDYLSDKHLGKISEDESSGLSHKSGSGEMTSEGSHTKDVASTDSDSVLNYRDGSTRLSL   1021
Bombys        ---------------------------------------------DSGV-----------   846
Drosophila    TMASSTAS--------------------------------QGSRPDTGIST---------   1020
Apis          TQTVVTFKHNDTNKHDQ------------------QTKNIMHNRQNIGVEE---------   945

Danio         SGMAVVDPISDTSSREWNDKS------QGARLPQSMSRYCVVCAYRTQLPIPSSWFLFYL   1042
Xenopus       SSSFLIHPLNNHINSNGPDHALAEDQFASPLL-------------------PEV--EKKQ   1030
Homo          DSDGNQNILSSTLTSKGNETI-ESIFKAEDLLPEAASLSENLDISKEETP-PVR--TLKS   1078
Mus           DDDGNHNPPSNPVTGKGIDAI-HSIFRAEDFLPEAASLSENPESSKEEAP-PAR--APKS   1077
Bombys        ------PPASSV------DVL----------L--AQS------LPAEEVPPP--------   868
Drosophila    QSQSQTQSISSASK----SAK----------S--AAA------RSGATDTVP--------   1050
Apis          EKV--LPPLRYA-L----DIT----------H--KHS------KGDSSSNTP--------   972
                                .                                *
```

```
Danio           FTNLHGR--LMQGKSQSSFSVSYKNMKKS----------------PSLQSLDDLSMDSYL     1084
Xenopus         LVDTSGIPKERCLPN---LSVSYKNMKRS----------------PSQFSLDNISIDSNL     1071
Homo            QSSLSGKPKERCPPNLAPLCVSYKNMKRS----------------SSQMSLDTISLDSMI     1122
Mus             QTSLSAKSKERCPPSPAPLSVSYKNMKRS----------------ASQVSLDTLSLDSMV     1121
Bombys          -------------ASPGLNFGGFSTMRRGFNSLVTSIDSALTRDDTRSDA------ASTA     909
Drosophila      -------------SLTKEINSGLLSMKKGFSSFMTSIDSAIKSGTPNDDASDTFSIQSDI     1097
Apis            -------------FIPNNFNVGLSSMKKGLSNLMTSIDSALKA-SPEDGSSDTVSIRSDV     1018
                                     .  .*::.                    :       *

Danio           LEDCDSYSLLDRDDVS----ISGFKEALNERI--------STDGSVTLAQEADEAQSPDA     1132
Xenopus         LDEQMIES----DGSD----HL---EVGMEDFSSLNYPCTAETSSVELRNEEYCVSSPDA     1120
Homo            LEEQLLES----DGSD----SHMFLEKGNKKNSTTNYRGTAESVNAGANLQNYGETSPDA     1174
Mus             LEEQ-AES----DGSD----SHVLLGKAMKRNSNTSCQSPAESVNTSANTQTCGEASPEA     1172
Bombys          SSDSDRYVVVGLAAESPDDADLAFREFE------HG----RASSAVEVAAEVVER-S---     955
Drosophila      SSDSDNFANVLGDDKTMDCMDVMFRLNP---FTTDN----MKASPVEVASEVYEE-QPSS     1149
Apis            SSDSENYVLVSLQDQ--EKLDTMFSVD-------NS----IRVAAVEEASEVVEE-TPDT     1064
                 .:                                          .    :

Danio           -------ISAASQSIDEPMKDLVSVLVMKVYSVCCSLDLKGDDTAVALEVGRVQPNQLGN     1185
Xenopus         -------ISA--ETSQDSRQDLMSVLVLKVVGINCGIDIKGEDATICLQINRVVPNQLGN     1171
Homo            -------ISTNSEGAQENHDDLMSVVVFKITGVNGEIDIRGEDTEICLQVNQVTPDQLGN     1227
Mus             -------VSTNSEGTQENRDDLMSVVVFRITGVNGEIDIRGEDTEVCLQVNQVTPSQLGN     1225
Bombys          -----SSPSDHSVTSSCRRRDVISTSTWRLNNIHVVHQSANGSSRLRLAADDLVSDECTA     1010
Drosophila      YKTNMSSPSEPSEGSTWRRRDLVSMATFRLTTVELIRQQEGPKSSVRLQVAAVSCDECGA     1209
Apis            -------QSEKSMDSVCKRKDIVSMITFKLSKVEFIQQSFGYASSIKIQISNIGNDECSS     1117
                        *           *::*  . ::  :    :  .  : : :    :  .:

Danio           VSLRQYLSNRSLGS-----GSG---SDPSAVLLNPEVQVRLESGPSAAV-----------     1226
Xenopus         VSVWQYLNSRNTGS-----DQK---STTDERKSSPEISLRLEIGPSARR-----------     1212
Homo            ISLRHYLCNRPVGS-----DQK---AVI-HSKSSPEISLRFESGPGAVI-----------     1267
Mus             VSLRHYLGNRPVGS-----DQK---AII-HPKSSPEISLRFESGPGAVV-----------     1265
Bombys          IPWDEFQ-------NKFSMRARAWTEPAIDEDSELKPR----ETPNVAARLTR-TELSRT     1058
Drosophila      IPWDELQIARQANKTKFGARCKAWNLAPYNPEAPPCIRMRLEETLNMPKEIEGI------     1263
Apis            IPWDEFQVKK---KTKFSARSRGWVELPSDSNCGSCIKLRLDHDLKCKSDSWKLSQASRT     1174
                :   .
```

```
Danio           --------------------------------HSPLAEQNGFLQCRLQAFNTDFLITSLR    1254
Xenopus         --------------------------------HSPLAAENGFLQCNVSNFSSEFLTSTLA    1240
Homo            --------------------------------HSLLAEKNGFLQCHIENFSTEFLTSSLM    1295
Mus             --------------------------------HSLLAEKNGFLQCHIENFTTEFLTSSLL    1293
Bombys          LN--------------------------ENGEATGLAPYEELLEARVRDLSLALNMSTAL    1092
Drosophila      -----------------------------IDRKRIQSWITHHAEIRVKDINMDLSMSTVI    1294
Apis            INNKNLYLSRNENQNNITEQDVDVCNIDIHNKQSVLDLFEDKLEVIITNISMALSMSSVS    1234
                                                           :  :  :.  :  ::

Danio           NLALFLEDDSASQVLPMEITIKDTHVNLKDDGPRDNISEPEPSPISVHIHNLLIHRQDDG    1314
Xenopus         NIHHFVEEDSVPEIMPMKIQVQNARIHLQDDSPRNNNTDPDPEPVVLNIENLFVERRDDG    1300
Homo            NIQHFLEDETVATVMPMKIQVSNTKINLKDDSPRSSTVSLEPAPVTVHIDHLVVERSDDG    1355
Mus             NIQHFLEDETVATVMPMKIQVSNTKINLKDDSPRGSTVSLQPSPVTVHIDRLVVERSDDG    1353
Bombys          ALSEFIEDEVIAPPMPLEVLIENVKVHLVEDRPVRSILSPPPQPLDIDLTTLRVTRASDG    1152
Drosophila      GLGDLAEDEVISPPMPLTVNLENVRINLLEDRPPVNITSPGPIPINLCIGRMRLERDQSG    1354
Apis            GLTDLTEDEIIPRPIPLQVYLESISLRLNEDRPPNNITSPGPIPIDLNIAKLKIIRDANG    1294
                 :  : *::     :*: : :..  :.* :* *  .  .  * *: : :  : : *  .*

Danio           SFSIGGAERAVDSQLQTAGPVNDSRLS------------SVPEVPVGVKATQTAPLSPTS    1362
Xenopus         SFWIRGSPDTSLNSPWNHKETSSILQLDCSKSHSTNSKSKTSKFTQTISEHTSLPEIP--    1358
Homo            SFHIRDSHMLNTGNDLKENVKSDSVLLTSGKYDL----KKQRSVTQA---TQTSPGVPWP    1408
Mus             SFHIRDSHLFNTGTDFKDGASSDSVVRTRGMCDV----RMHSSVTQA---TQTSPEVPLP    1406
Bombys          VLRLGPPLSTPSTVASPST-----------PQPN----PELDEARAAIDSLN-KENEELR    1196
Drosophila      LLNIQPIDTNMSDAQHQAL-G--SALFGAPRERD----RELLSMQLVMQQMK-LDNDQLR    1406
Apis            VFHIEPVVNLLSRSNSLVTLTSNDTQIAQNINHE----MELNVLRQSSKQLK-LDNEQLR    1349
                 : :

Danio           ---------PGPSSREQLLVEENECLKLELSKAKMALAEAQMEKDSLQHQMKTLKLTSGG    1413
Xenopus         DVINNQ-AWKAAGISKEQLVEENECLKQELAKTKMALAESHMERDRLLHQLKRVHIEK--    1415
Homo            SQSANF-PEFSFDFTREQLMEENESLKQELAKAKMALAEAHLEKDALLHHIKKMTVE---    1464
Mus             SQSANF-L----DITREQLMEENECLRQRLAQAKMELAEAHSARDELLHQMKRMGL----    1457
Bombys          KRLLTLSRIADDNRELRAKVEEAAALRQCVHKAQQEAVSLLADKQELLETVRALQEQAGC    1256
Drosophila      RQLVDSKV--NTDNYRHKTKQEADVLRSYLKAAQDDISILLEEKKALLDTIRSLQVQLTS    1464
Apis            SRLNALEKLSEENAKLIRIKEESNVIKSHLSAAQEDIQLLLKEKRALQETITQLENRIIG    1409
                            .       :*   ::  :  ::         :  * . :  :
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Danio           SNS------------ 1416
Xenopus         --------------- 1415
Homo            --------------- 1464
Mus             --------------- 1457
Bombys          RGKR----------- 1260
Drosophila      SNMSRKSDGNR---- 1475
Apis            SGLGSGTRASWSSKR 1424
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Figure 5. Alignment of human UHRF1BP1L protein with similar protein from selected vertebrates and invertebrates.

Clustal Omega multiple sequence alignment of *Homo sapiens* UHRF1-binding protein 1-like protein (Homo is *Homo sapiens* NP_055869.1) with the Zebrafish uhrf1bp1l (Danio is *Danio rerio* NP_001093475.1), house mouse Uhfr1bp1l (Mus is *Mus musculus* NP_083442.2), frog uhrf1bp1l (Xenopus is *Xenopus laevis* NP_001084948.1), the honey bee (Apis is *Apis mellifera* XP_006559910.2), silk moth (Bombys is *Bombys mori* XP_012551608.1), and Dmel/CG34126 (Drosophila is *Drosophila melanogaster* NP_001260065.1). The domains were identified using the CD-search tool of NCBI Conserved Domain Database Search (CDD) and Pfam. "*" (Asterisk) indicates the residues that are identical, ":" (Colon) indicates the strongly similar, "." (Period) indicates the weakly similar. Colors show the chemical nature of amino acids. Red is small hydrophobic (including aromatic Y), Blue is acidic, Magenta is basic, and Green is basic with hydroxyl or sulfhydryl or amine groups. Grey is unusual amino acids. Black and yellow represent the shared domains of proteins. The proteins of the seven species share 150 identical, 221 highly conserved, and 77 less conserved amino acids.

Analysis of the consequences of altered expression in the developing eye

Effects of overexpression and RNA interference of *CG34126* in eye phenotypes

The compound eye development of *D. melanogaster* compound eye is very precise and regular. The development and organization of each ommatidium and its array is tightly controlled (Thomas & Wassarman, 1999). Each eye is composed of approximately 700 to 800 ommatidia under normal conditions. The eye is a photoreceptor organ, controlled by neurons. If any neurodegeneration occurs, it may result changes to ommatidia area, ommatidia and bristle numbers or appearance of rough eye. To observe any type of phenotypic changes in eyes controlled by neurons, we did biometric analysis to determine whether overexpression or RNA-interference of *CG34126* has any effect on the development of specialized neurons in the visual unit. The eye specific transgenic line *GMR-Gal4* was used to express *CG34126* transgenes. A summary of ommatidia and bristle number is demonstrated in Table 3. Analysis of the scanning electron micrographs is showed in the Figure 6.

Scanning electron micrographs of eyes through overexpression or inhibition indicated that there were no significance changes in the ommatidium number (Figure 7A) and the bristle numbers compare to control (Figure 7B), when these transgenes were expressed in the eyes by *GMR-GAL4* (Table 3A and 3B).

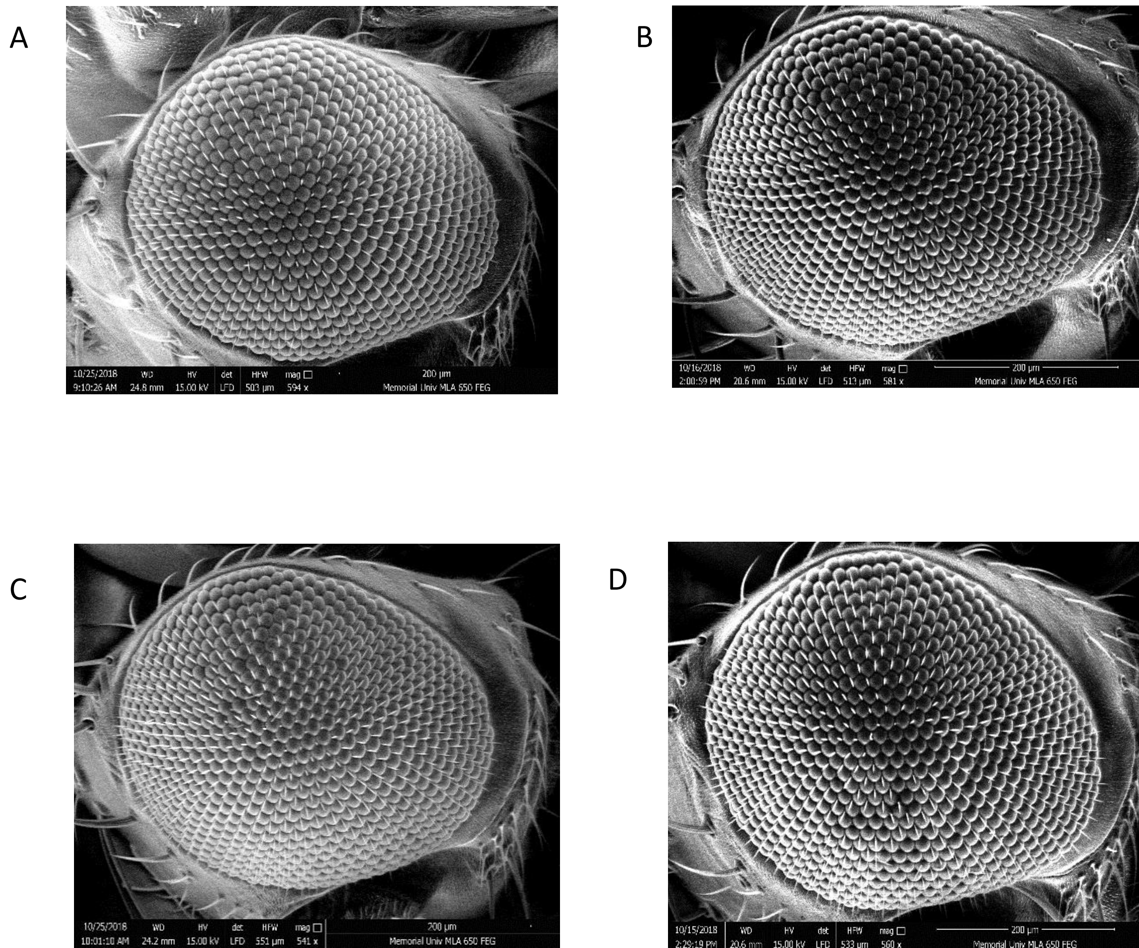

Figure 6. Eye of critical class of *Drosophila melanogaster* with altered *CG34126* expression visualised by scanning electron microscopy.

A) *GMR-Gal4/UAS-lacZ*; B) *GMR-Gal4/ UAS-CG34126*^{EY}; C) *GMR-Gal4/ UAS-CG34126-RNAi*^{GD}; D) *GMR-Gal4/ UAS-CG34126-RNAi*^{KK}. There is no significant differences in the number of ommatidia or inter-ommatidial bristles. Images were captured with a FEI MLA 650 Scanning Electron Microscope.

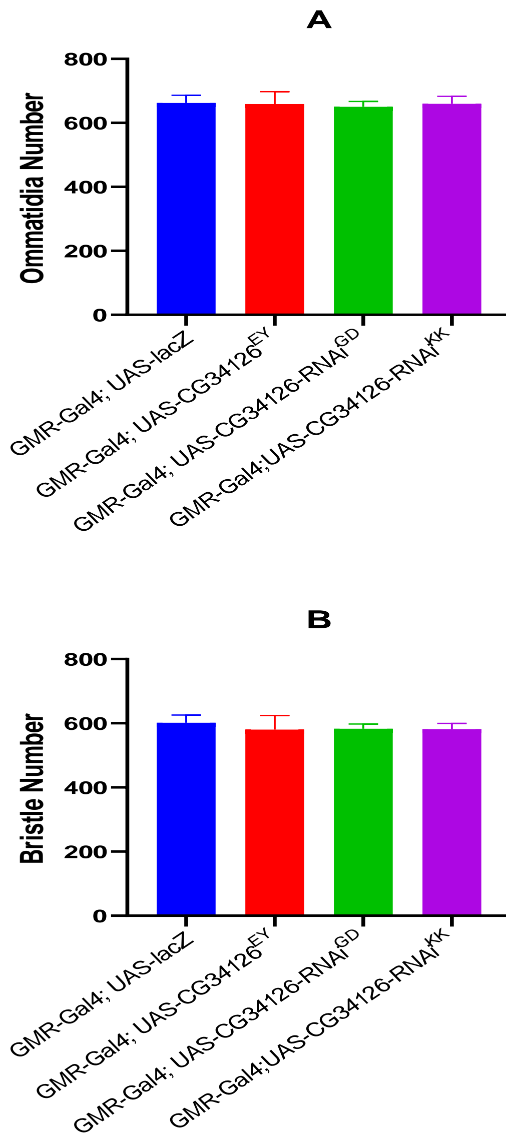

Figure 7. Biometric analysis of *Drosophila melanogaster* eye with altered *CG34126* expression. (A) Ommatidium number and (B) bristle number.

There was no significance change in the ommatidia numbers and bristle when overexpression and knockdown of *CG34126* genes were analyzed in the eye. All counts compared to a *UAS-lacZ* control, comparisons were measured using a one-way ANOVA and significance was tested by unpaired t-test ($P<0.05$ and 95%CI), number of eyes=10.

Table 3: Biometric analysis of the directed overexpression and RNA interference of *CG34126* in the developing Drosophila eye.

A. Ommatidia number

Genotypes	Mean number Ommatidium	Mean difference ± SEM	Significance (P-value compare to control)	95% confidence interval
GMR-Gal4; UAS-lacZ	662.3			
GMR-Gal4; UAS-CG34126^{EY}	659.2	-3.100 ± 14.45	0.8326 No	-33.46 to 27.26
GMR-Gal4; UAS-CG34126-RNAi^{GD}	650.0	-12.30 ± 9.408	0.2075 No	-32.07 to 7.466
GMR-Gal4;UAS-CG34126-RNAi^{KK}	659.9	-2.400 ± 10.59	0.8232 No	-24.64 to 19.84

B. Inter-ommatidia bristle number

Genotypes	Mean number Bristle	Mean difference ± SEM	Significance (P-value compare to control)	95% confidence interval
GMR-Gal4; UAS-lacZ	601.8			
GMR-Gal4; UAS-CG34126^{EY}	580.1	-21.70 ± 15.99	0.1915 No	-55.29 to 11.89
GMR-Gal4; UAS-CG34126-RNAi^{GD}	583.2	-18.60 ± 8.886	0.0508 No	-37.27 to 0.06973
GMR-Gal4;UAS-CG34126-RNAi^{KK}	581.9	-19.90 ± 9.500	0.0506 No	-39.86 to 0.05876

Overexpression and inhibition of *CG34126* gene in selected neurons

A prominent feature of PD is the age-dependent degeneration of dopaminergic neurons. The selective demise and degeneration of these dopaminergic neurons lead us to investigate the effects of *UHRF1BP1L* on these neurons. To observe the phenotypic (ageing and climbing) effects of *CG34126* transgenes on the DA and motor neuronal loss of *Drosophila*, different control and experimental lines both were overexpressed and silenced via RNA interference and directed by *D42-Gal4, Ddc-Gal4*, and *TH-Gal4* transgenes. The ageing analysis was carried out simultaneously with the climbing assay in order to determine the changes in the climbing ability as a result of premature senescence.

Lifespan analysis

The effects of loss-of-function and gain-of-function of *CG34126*

To investigate the effects of gain-of-function and loss-of-function of *CG34126* on longevity, the *Gal4*-expressing lines *D42-Gal4*, *Ddc-Gal4* and *TH-Gal4* were used to cross with control *UAS-lacZ* and experimental lines to direct the expression of the *CG34126* transgenes in the fly DA neurons. There was a significance difference in the lifespan of flies when *CG34126* transgenes were overexpressed and inhibited in the motor neurons directed by *D42-Gal4* and survival curves were demonstrated in Figure 8. The inhibition of *UAS-CG34126*-RNAi^{GD} demonstrated a significant reduction in longevity of flies compared to *lacZ* control. The median lifespan for *UAS-CG34126-RNAi^{GD}* line was 56 days verses 64 days for the control (Table 4). The log-rank test showed that the longevity curve was significantly different (p<0.0001) from the control curve. Overexpression of

UAS-CG34126^{EY} and another inhibition line *UAS-CG34126-RNAi*^{KK} showed no significant decrease in mean lifespan compared to *lacZ* control, and median lifespans of both lines were 62 days. Both the loss-of-function of *UAS-CG34126-RNAi*^{GD} and overexpression of *UAS-CG34126*^{EY} significantly reduced survival ability of flies (Figure 9) compared to *lacZ* control using *Ddc-Gal4*, which had mean lifespans of 62, 62 and 70 days (Table 5), respectively. The mean life span of *UAS-CG34126-RNAi*^{KK} transgenic flies was 72 days, which was not statistically significant verses to the control. Statistical analysis of longevity is summarized in Table 5. Like the *D42-Gal4* and *Ddc-Gal4* directed expression lines, inhibition of *UAS-CG34126-RNAi*^{GD} in DA neurons by *TH-Gal4* resulted in a prominent reduction of longevity compared to control (Figure 10) and statistical analysis from table 6 showed median lifespan of this line was 70 days verses 76 days for the *lacZ*-expressing control. The log-rank test showed that the longevity curve was significantly different ($p<0.0001$) from the control curve. The overexpression of *UAS-CG34126*^{EY} and inhibition line *UAS-CG34126-RNAi*^{KK} had no significant decrease in mean lifespan compared to the *lacZ* control, and median lifespan of these overexpressed and inhibited lines were 74 and 78 days (Table 6). It was evident that loss-of-function of *CG34126* in fly dopaminergic neurons leads to a reduction in lifespan, which is characteristic of PD-like phenotype, whereas overexpression except *Ddc-Gal4* directed flies are aligned to normal flies.

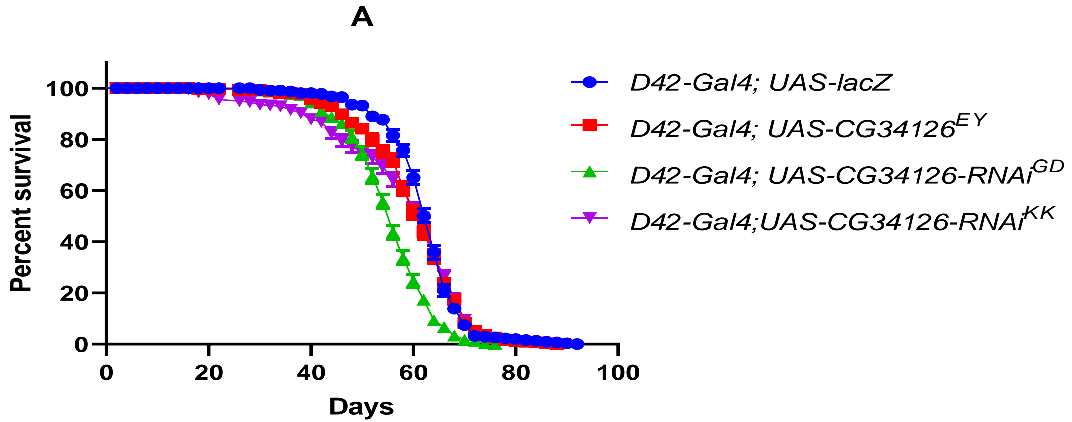

Figure 8. Longevity curves of flies with altered expression of *CG34126* in the motor neurons.

Inhibition of *UAS-CG34126-RNAi*^{GD} directed by *D42-Gal4* results in decreased survival compared to the control *UAS-lacZ*. *UAS-CG34126-RNAi*^{KK} expression in dopaminergic neurons causes no significant decrease in mean lifespan compared to *lacZ* control, while overexpression of *UAS-CG34126*^{EY} results almost same as *lacZ* control. Longevity was shown as a percent survival (P<0.05 as determined by the Mantel-Cox Log Rank test). Significance was determined at 95%, at a P value less than or equal to 0.05 with Bonferroni correction. Error bar represents standard error and see Table 4 for n values.

Table 4: Comparison of survival of flies with altered expression of *CG34126* by *D42-GAL4* by (Mantel-Cox) Log-rank test.

Genotypes	Number of flies	Mean survival Days	P-value (Bonferroni correction) compare to control	Significance
D42-Gal4; UAS-lacZ	309	64	N/A	N/A
D42-Gal4; UAS-CG34126^{EY}	303	62	<0.2816	No
D42-Gal4; UAS-CG34126-RNAi^{GD}	300	56	<0.0001	Yes
D42-Gal4;UAS-CG34126-RNAi^{KK}	296	62	<0.2224	No

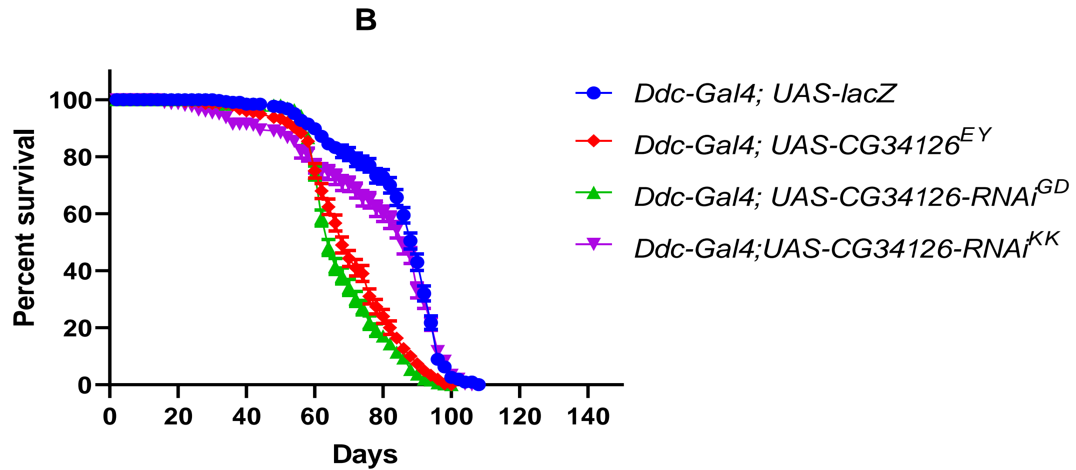

Figure 9. Longevity curves of flies with altered expression of *CG34126* in the dopaminergic neurons.

Knockdown of *UAS-CG34126-RNAi*^{GD} and overexpression of *UAS-CG34126*^{EY} in the DA neurons directed by *Ddc-Gal4* resulted in decreased survival compared to the control *UAS-lacZ*. *UAS-CG34126-RNAi*^{KK} expression in dopaminergic neurons caused no significant decrease in mean lifespan compared to *lacZ* control. Longevity was shown as a percent survival ($P<0.05$ as determined by the Mantel-Cox Log Rank test). Significance was determined at 95%, at a P value less than or equal to 0.05 with Bonferroni correction. Error bar represents standard error and see Table 5 for n values.

Table 5: Comparison of survival of flies with altered expression of *CG34126* by *Ddc-GAL4* by (Mantel-Cox) Log-rank test.

Genotypes	Number of flies	Mean survival Days	P-value (Bonferroni correction) compare to control	Significance
Ddc-Gal4; UAS-lacZ	291	70	N/A	N/A
Ddc-Gal4; UAS-CG34126^{EY}	302	62	<0.0001	yes
Ddc-Gal4; UAS-CG34126-RNAi^{GD}	308	62	<0.0001	Yes
Ddc-Gal4;UAS-CG34126-RNAi^{KK}	303	72	<0.0667	No

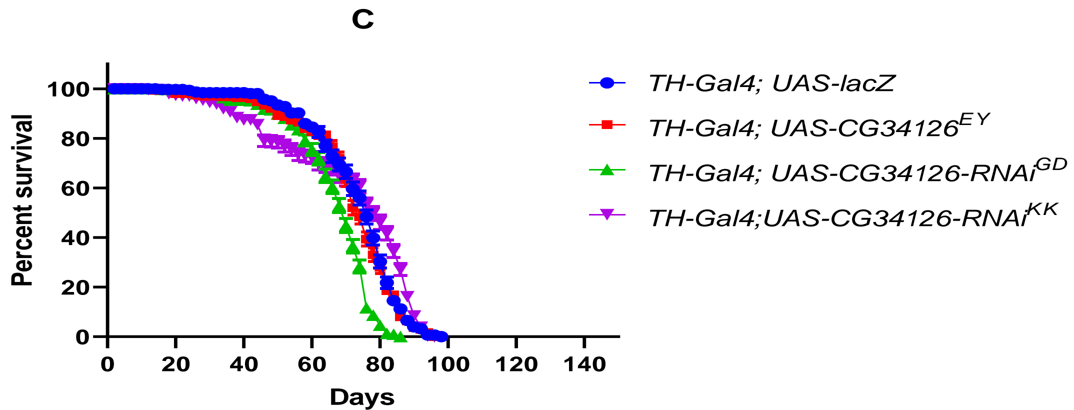

Figure 10. Longevity curves of flies with altered expression of *CG34126* in the motor neurons.

Overexpression of *UAS-CG34126*^{EY} and Inhibition of *UAS-CG34126-RNAi*^{KK} in the dopaminergic neurons directed by *TH-Gal4* had no prominent effect on lifespan compared to the control *UAS-lacZ* but knockdown of *UAS-CG34126-RNAi*^{GD} in dopaminergic neurons causes significant decrease in mean lifespan compared to *lacZ* control. Longevity was shown as a percent survival (P<0.05 as determined by the Mantel-Cox Log Rank test). Significance was determined at 95%, at a P value less than or equal to 0.05 with Bonferroni correction. Error bar represents standard error and see table 6 for n values.

Table 6: Comparison of survival of flies with altered expression of *CG34126* by TH-*GAL4* by (Mantel-Cox) Log-rank test.

Genotypes	Number of flies	Mean survival Days	P-value (Bonferroni correction) compare to control	Significance
TH-Gal4; UAS-lacZ	303	76	N/A	N/A
TH-Gal4; UAS-CG34126^{EY}	299	74	<0.3502	No
TH-Gal4; UAS-CG34126-RNAi^{GD}	301	70	<0.0001	Yes
TH-Gal4;UAS-CG34126-RNAi^{KK}	306	78	<0.1922	No

Climbing analysis of the control and experimental lines

The effects of loss-of-function and gain-of-function of *CG34126*

The climbing analysis was carried out to investigate whether the interference of expression and overexpression of *CG34126* has any effect on dopaminergic neurons, which would have an effect on fly mobility. The effects of gain-of-function and loss-of-function of *CG34126* on climbing ability were investigated using the lines *D42-Gal4, Ddc-Gal4* and *TH-Gal4* in order to express the transgenes in the fly dopaminergic, motor and other neurons. Inhibition of *CG34126* utilizing all three *Gal4* directed lines significantly decreased climbing ability compared to the *lacZ*-expressing control (Figure 11, 12, and 13, Table 7, 8, and 9). The 95% confidence interval for *D42-Gal4/ UAS-CG34126-RNAi^{GD}* and *D42-Gal4/ UAS-CG34126-RNAi^{KK}* were 0.04441 to 0.06170 and 0.02883 to 0.03828 (Table 7), for *Ddc-Gal4/ UAS-CG34126-RNAi^{GD}* and *Ddc-Gal4/ UAS-CG34126-RNAi^{KK}* were 0.008707 to 0.03684 and 0.03023 to 0.04347 (Table 8), and for *TH-Gal4/ UAS-CG34126-RNAi^{GD}* and *TH-Gal4/ UAS-CG34126-RNAi^{KK}* were 0.02651 to 0.04898 and 0.03056 to 0.04281 (Table 9). These climbing curves are significantly different from the control curves with 95% confidence intervals at 0.04337 to 0.06121 for *D42-Gal4*, 0.06331 to 0.09724 for *Ddc-Gal4*, and 0.04581 to 0.06941 for *TH-Gal4*. Overexpression of *CG34126* using *D42-Gal4* (Figure 11) and *TH-Gal4* (Figure 13) did not show significant decline or increase in climbing ability compared to the *lacZ*-expressing control except *Ddc-Gal4/ UAS-CG34126^{EY}* with 95% confidence interval of 0.03723 to 0.05627, which indicates less climbing ability compare to standard line (Table 8). The 95% confidence interval for *D42-Gal4/ UAS-CG34126^{EY}* was 0.03144 to 0.04416, and *TH-Gal4/ UAS-CG34126^{EY}* was 0.03368 to 0.06038.

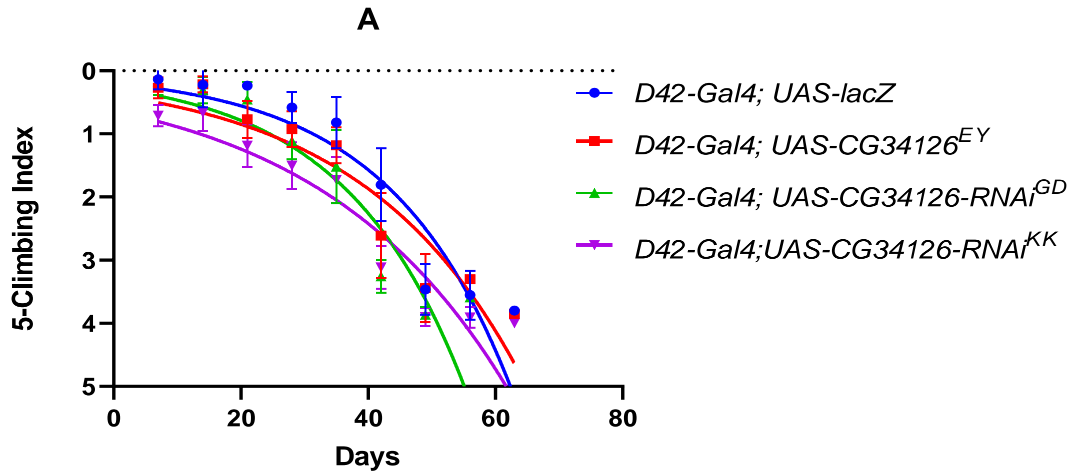

Figure 11. Climbing ability over time of flies with altered expression of *CG34126* in motor neurons.

Both inhibition of *UAS-CG34126-RNAi*^{GD} and *UAS-CG34126-RNAi*^{KK} in the motor neurons directed by *D42-Gal4* results in reduced climbing ability as determined by non-linear fitting of the climbing curves and comparing at 95% confidence intervals. Overexpression *UAS-CG34126*^{EY} had no significant effect on locomotor ability compared to the control. Error bar represents standard error and n=50.

Table 7: Comparison of climbing ability of flies with altered expression of *CG34126* by *D42-GAL4.*

Genotypes	Standard error	95%CI	R square	P value	Significance
D42-Gal4; UAS-lacZ	0.004986	0.04337 to 0.06121	0.8290		
D42-Gal4; UAS-CG34126^{EY}	0.003430	0.03144 to 0.04416	0.8209	<0.0656	No
D42-Gal4; UAS-CG34126-RNAi^{GD}	0.004775	0.04441 to 0.06170	0.8614	<0.0001	Yes
D42-Gal4;UAS-CG34126-RNAi^{KK}	0.002494	0.02883 to 0.03828	0.8677	<0.0001	Yes

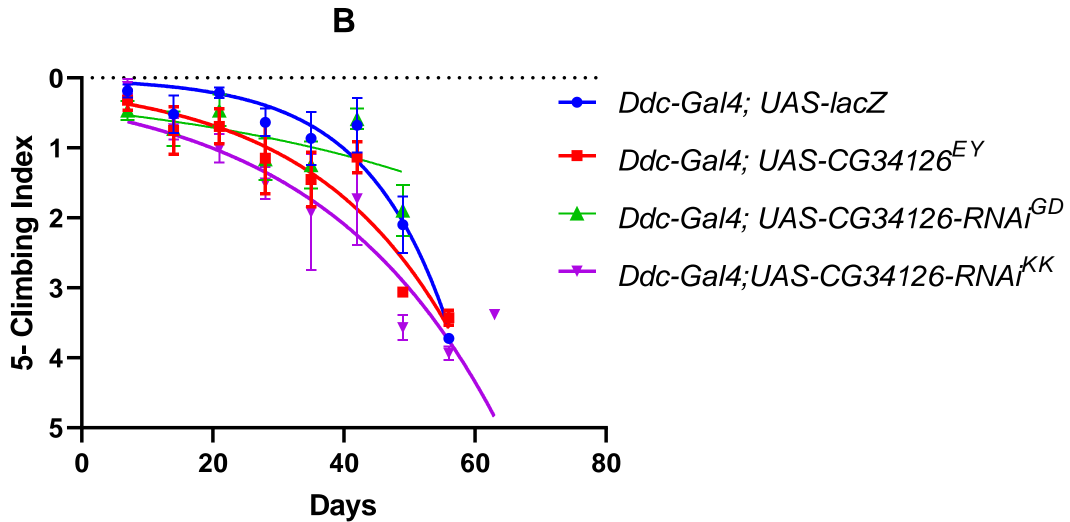

Figure 12. Climbing ability over time of flies with altered expression of *CG34126* in motor neurons.

Knockdown of *UAS-CG34126-RNAi*^{KK} and *UAS-CG34126-RNAi*^{GD} , and overexpression of *UAS-CG34126*^{EY} directed by *Ddc-Gal4* decreased climbing ability over time compared to the control *lacZ* when expressed by dopaminergic neurons specific transgene as obtained by non-linear fitting of the climbing curves. Error bar represents standard error and n=50.

Table 8: Comparison of climbing ability of flies with altered expression of *CG34126* by *Ddc-GAL4.*

Genotypes	Standard error	95%CI	R square	P value	Significance
Ddc-Gal4; UAS-lacZ	0.007273	0.06331 to 0.09724	0.8449		
Ddc-Gal4; UAS-CG34126^{EY}	0.004507	0.03723 to 0.05627	0.8044	<0.0001	Yes
Ddc-Gal4; UAS-CG34126-RNAi^{GD}	0.006747	0.008707 to 0.03684	0.2776	<0.0001	Yes
Ddc-Gal4;UAS-CG34126-RNAi^{KK}	0.003491	0.03023 to 0.04347	0.8073	<0.0001	Yes

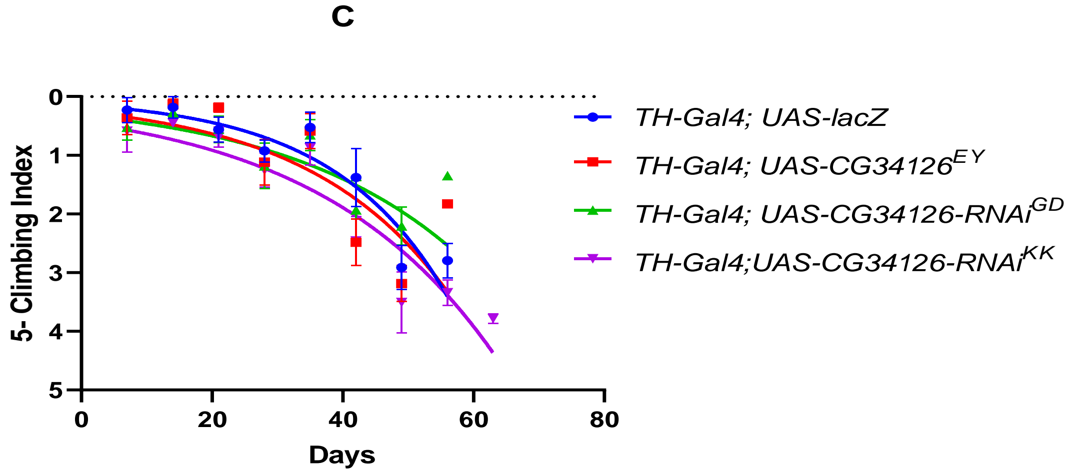

Figure 13. Climbing ability over time of flies with altered expression of *CG34126* in motor neurons.

Overexpress of *UAS-CG34126*^{EY} had no significant effect on locomotor ability compared to the control when expressed by *TH-GAL4* transgene, but inhibition of both *UAS-CG34126-RNAi*^{KK} and *UAS-CG34126-RNAi*^{GD} caused decreased climbing ability compared to control *lacZ* as obtained by non-linear fitting of the climbing curves and analyzing at 95% confidence intervals. Error bar represents standard error and n=50.

Table 9: Comparison of climbing ability of flies with altered expression of *CG34126* by *TH-GAL4.*

Genotypes	Standard error	95%confidence interval	R^2	p-value	Significance
TH-Gal4; UAS-lacZ	0.006012	0.04581 to 0.06941	0.8077		
TH-Gal4; UAS-CG34126^{EY}	0.007513	0.03368 to 0.06038	0.6456	0.3596	No
TH-Gal4; UAS-CG34126-RNAi^{GD}	0.08034	0.02651 to 0.04898	0.6225	0.0358	Yes
TH-Gal4;UAS-CG34126-RNAi^{KK}	0.003243	0.03056 to 0.04281	0.8238	0.0008	Yes

Expression of *CG34126* in the *park* interference model of PD

The co-expression of the experimental lines with *park (Ddc-GAL4/CyO; UAS-park-RNAi/TM3)* was carried out to determine the effects of overexpression and inhibition of the *UHRF1BP1L* homologue *CG34126* in the dopaminergic neurons. Both ageing and climbing ability were analyzed and compared to results obtained in *park-* RNA interference expressing control flies.

Longevity analysis of the control and experimental lines in the *park* model

The co-expression of *CG34126* transgenes with *parkin* demonstrated various survival curves. Inhibition of *UAS-CG34126-RNAi*^{GD} and *UAS-CG34126-RNAi*^{KK} had a reduction in survival ability when they crossed with *Ddc-Gal4;UAS-park-RNAi* (Figure 14 and Table 10). Median survival was 40 days for *UAS-CG34126-RNAi*^{GD}, which prominently varied compare to the 46 days of control *lacZ* when both lines were directed by dopaminergic transgene *Ddc-Gal4;Uas-park-RNAi*. On the other hand, there was no statistical significance between the lifespan capability of *UAS-CG34126-RNAi*^{KK} and *UAS-lacZ* control when they crossed with *Ddc-Gal4;UAS-park-RNAi*.

Overexpression of *CG34126* in dopaminergic neurons rescues *UAS-park-RNAi* mediated loss of longevity

Strikingly, the co-expression of *UAS-CG34126*^{EY} with *park-RNAi* showed an improvement in the lifespan of flies when expressed in the dopaminergic neurons (Figure 14 and Table 10). Median lifespan was 54 days for the flies generated from the crossing of *UAS-CG34126*^{EY} and *Ddc-Gal4;UAS-park-RNAi,* which was significantly higher than the median lifespan (46 days) of the control *lacZ* and *Ddc-Gal4;UAS-park-RNAi* mediated flies.

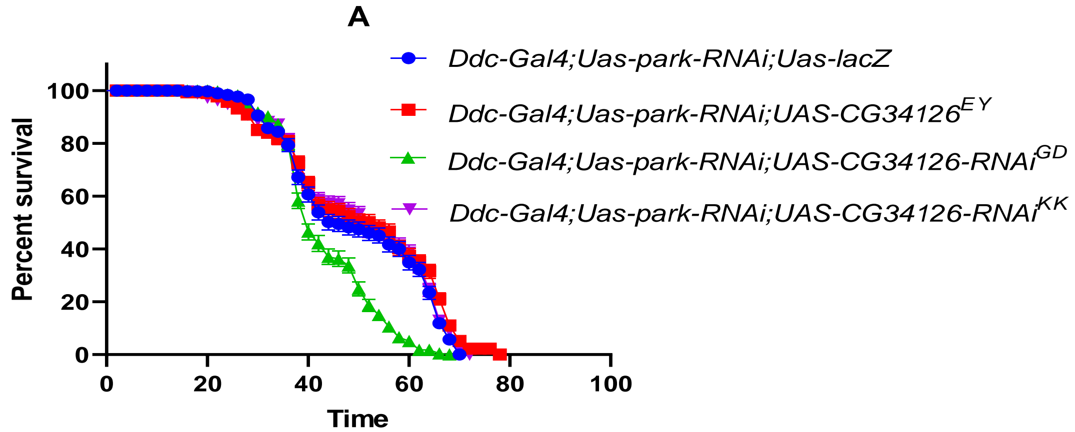

Figure 14. Longevity of flies with inhibition of *park* along with altered expression of *CG34126* in *Ddc-GAL4* expressing neurons.

Inhibition of *UAS-CG34126-RNAi*^{GD} had reduced survival rate compared to the control *UAS-lacZ* and inhibition of *UAS-CG34126-RNAi*^{KK} did not significantly differ with the control. The longevity of flies increased when *UAS-CG34126*^{EY} co-expressed with *Ddc-Gal4;UAS-park-RNAi*. Genotype Longevity was shown as a percent survival (P<0.05 as determined by the Mantel-Cox Log-Rank test). Significance was determined at 95%, at a P value less than or equal to 0.05 with Bonferroni correction. Error bar represents standard error and see table 10 for n values.

Table 10: Comparison of survival of flies with inhibition of *park* along with altered expression of *CG34126* in *Ddc-GAL4* expressing neurons by (Mental-Cox) Log-rank test.

Genotypes	Number of flies	Mean survival Days	P-value (Bonferroni correction) compare to control	Significance
Ddc-Gal4;UAS-park-RNAi;UAS-lacZ	295	46	N/A	N/A
Ddc-Gal4;UAS-park-RNAi;UAS-CG34126^{EY}	305	54	<0.0081	yes
Ddc-Gal4;UAS-park-RNAi;UAS-CG34126-RNAi^{GD}	272	40	<0.0001	Yes
Ddc-Gal4;UAS-park-RNAi;UAS-CG34126-RNAi^{KK}	295	52	<0.2151	No

Climbing analysis of the control and experimental lines in the *UAS-park-RNAi* model.

Loss of locomotor power is one of the prominent symptoms of PD phenotypes as well as *parkin* RNAi mediated model of PD. So, it is worthwhile to detect the contributing role of *CG34126* gene alteration in the *park* inhibition models. To investigate the probable effect in the dopaminergic neurons two inhibition lines *UAS-CG34126-RNAi*^{GD} and *UAS-CG34126-RNAi*^{KK} and one overexpression line *UAS-CG34126*^{EY} were crossed with the derivate line *Ddc-Gal4;UAS-park-RNAi.*

CG34126 inhibition exacerbates climbing dysfunction in the *UAS-park-RNAi* model

Inhibition of both *CG34126* and *park* in the *Drosophila* showed a reduced climbing ability over time with the control flies (Figure 15). The 95% confidence interval for the flies produced from crossing of *UAS-CG34126-RNAi*^{GD} with *Ddc-Gal4;UAS-park-RNAi*, *UAS-CG34126-RNAi*^{KK} with *Ddc-Gal4;UAS-park-RNAi* and *UAS-lacZ* with *Ddc-Gal4;UAS-park-RNAi* were 0.03366 to 0.05601, 0.02466 to 0.03611 and 0.03010 to 0.04512 respectively (Table 11).

CG34126 overexpression rescues climbing ability in *UAS-park-RNAi* model

Flies generated by crossing of *UAS-CG34126*^{EY} and *Ddc-Gal4;Uas-park-RNAi* represented gradually better climbing ability compare to flies produced from crossing of control *UAS-lacZ* and *Ddc-Gal4;Uas-park-RNAi* (Figure 15).

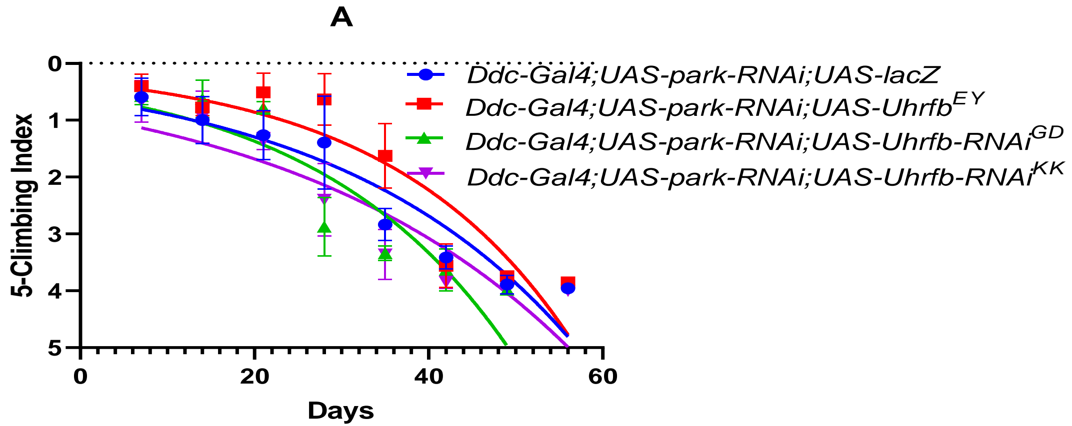

Figure 15. Climbing ability over time of flies with inhibition of *park* along with altered expression of *CG34126* in *Ddc-GAL4* expressing neurons.

Inhibition of *UAS-CG34126-RNAi*^{KK} and *UAS-CG34126-RNAi*^{GD} directed by *Ddc-Gal4;UAS-park-RNAi* decreased climbing ability over time compared to the control *lacZ* as obtained by non-linear fitting of the climbing curves and analyzing at 95% confidence intervals ($P<0.05$). But co-expression of *UAS-CG34126*^{EY} and *park*-RNAi had significant effect in increasing locomotor ability compared to the control. Error bar represents standard error and n=50.

Table 11: Comparison of climbing ability of flies with inhibition of *park* along with altered expression of *CG34126* in *Ddc-GAL4* expressing neurons by (Mental-Cox) Log-rank test.

Genotypes	Standard error	95%CI	R square	P value	Significance
Ddc-Gal4;UAS-park-RNAi;Uas-lacZ	0.003850	0.03010 to 0.04512	0.8008		
Ddc-Gal4;UAS-park-RNAi;UAS-CG34126^{EY}	0.004973	0.03880 to 0.05754	0.8077	<0.0383	Yes
Ddc-Gal4;UAS-park-RNAi;UAS-CG34126-RNAi^{GD}	0.005894	0.03366 to 0.05601	0.7432	<0.0480	Yes
Ddc-Gal4;UAS-park-RNAi;UAS-CG34126-RNAi^{KK}	0.003067	0.02466 to 0.03611	0.7966	<0.0366	Yes

Table 12: Summary of longevity and climbing over time results

Lifespan Analysis			
Different genotypes of *CG34126*	***D42-GAL4***	***Ddc- GAL4***	***TH-GAL4***
Overexpression	**No change**	**Decreased lifespan**	**No change**
Inhibition ***$RNAi^{GD}$***	**Decreased lifespan**	**Decreased lifespan**	**Decreased lifespan**
Inhibition ***$RNAi^{KK}$***	**No change**	**No change**	**No change**
Climbing Analysis			
Overexpression	**No change**	**Decreased climbing ability**	**No change**
Inhibition ***$RNAi^{GD}$***	**Decreased climbing ability**	**Decreased climbing ability**	**Decreased climbing ability**
Inhibition ***$RNAi^{KK}$***	**Decreased climbing ability**	**Decreased climbing ability**	**Decreased climbing ability**
Different lines of *CG34126* crossed with *Ddc-GAL4/CyO; UAS-park-RNAi/TM3*.			
Lifespan Analysis			
Overexpression		**Increased lifespan**	
Inhibition ***$RNAi^{GD}$***		**Decreased lifespan**	
Inhibition ***$RNAi^{KK}$***		**No change**	
Climbing Analysis			
Overexpression		**Increased climbing ability**	
Inhibition ***$RNAi^{GD}$***		**Decreased climbing ability**	
Inhibition ***$RNAi^{KK}$***		**Decreased climbing ability**	

Discussion

Parkinson Disease (PD) is initiated by a variety of genetic and environmental factors with an age-dependent demise of dopaminergic neurons, often associated with the aggression of the α-synuclein protein, is a common pathological condition (Botella *et al*., 2009). There are many cellular processes such as mitochondrial dysfunction, oxidative stress, endo-lysosomal dysfunction, Golgi body dysregulation, and dysregulation of protein-trafficking that contribute to the symptoms of PD. Internal functions of the mitochondria modify non-functional proteins or degrade damaged protein. Interestingly, the ubiquitin-proteasome system has control over mitochondrial functionality and their mutual interaction is linked to PD (Franz *et al.,* 2015). The gene selected for the study, *UHRF1BP1L*, has been shown to interact with mitochondrial dynamics by diminishing its number in affected neurons with altered morphology (Jansen *et al*., 2017). The UHRF1BP1L protein shares the Chorein-N domain with VPS13, which is a membrane trafficking protein. It might play an important role in both the integral surveillance of mitochondrial mechanisms and protein degradation. The *UHRF1BP1L* homologue *CG34126* in *Drosophila* has not been extensively studied to observe ectopic expression. This study aimed to examine various aspects of the *Drosphila melanogaster* homologue of *UHRF1BP1L*. *CG34126* was overexpressed and inhibited in dopaminergic, motor, and other neurons of *Drosophila melanogaster* to determine its effects on cell death and growth which eventually contribute to longevity, locomotor ability, and eye development to recapitulate the phenotypic symptoms of PD.

Sequence alignment of human *UHRF1BP1L* and its homologue *CG34126* in flies was carried out using the bioinformatics tool to identify similarity and identity. These two sequences showed more than 26.5% identity and 41.9% similarity in their amino acid sequences and they share the conserved domain Chorein-N and VPS13, indicating that *Drosophila melanogaster CG34126* is closely related and a functional homologue to human *UHRF1BP1L.* Alignment of *Drosophila CG34126* and human *UHRF1BP1L* with three invertebrate and vertebrate species further reinforces the evolutionary conservation of amino acid sequences. Same domains Chorein-N and VPS13 are well conserved among all the seven different protein sequences. The full-length VPS13 protein is a transmembrane protein with a presumed role in vesicle-mediated sorting and intracellular protein transport and it has a Chorein-N domain (Velayos-Baeza *et al*., 2004). Recent studies show that VPS13C regulates lipid trafficking between the endoplasmic reticulum and other membranous organelles. Dysfunction of lipid homeostasis due to mutation of VPS13C likely show clinical manifestations in patients with Parkinsonism (Kumar *et al.,* 2018). As the Chorein-N domain has been noted in both UHRF1BP1L and VPS13C protein (Mizuno *et al*., 2007) and its presence likely indicates that UHRF1BP1L has lipid transport modules alongside protein trafficking, which could affect PD pathology in a similar manner to VPS13C. However, another VPS35 protein has been linked to early-onset Parkinsonian-pyramidal syndrome due to an impairment of endosome-to-Golgi retrieval of membrane proteins (Zimprich *et al.,* 2011b; Vilariño-Güell *et al*., 2013). It can be inferred that *CG34126* plays a role in protein transferring dysfunction in PD. Furthermore, bioinformatic analysis was carried out to investigate the relationship between CG34126

protein and human UHRF1BP1, which is the only paralogue of human protein UHRF1BP1L. Alignment of human UHRF1BP1 and *Drosophila* CG34126 showed that the evidence of the conservation, such as 26.7% identical and 42.5% similar in amino acid sequence and is highly conserved in the Chorein-N and VPS13 domains. This indicates that similar to *UHRF1BP1L, CG34126* is closely related to *UHRF1BPI.* Pairwise sequence alignment of human and fly demonstrated FNIII, ID/GR, and CC domains were conserved within the same amino acid sequences of both proteins, though these domains were not identified in fly by the CDD and Pfam. Two available domains, Chorein-N and VPS13, in both species showed more than 50% similarity, which validates the study of PD in the fly genes. Moreover, the presence of these two domains reinforce the protein trafficking role of *CG34126* in PD pathogenesis.

Genetic expression studies have extensively used the *Drosophila* eye to study of neurodegeneration because of the conservation of key signalling pathways between human and fly, and the ease of quantifying degeneration of photoreceptor neurons associated with each ommatidium. In *Drosophila melanogaster,* eye development is tightly controlled during the organization of the ommatidial array (Thomas & Wassarman, 1999). The eye consists of specialized structures called sensory bristles, that provide the opportunity for neurogenesis examination and for detection of even slight abnormalities (Baker, 2001). Reduction in the number of ommatidia, bristle and ommatidia area suggests a reduction in cell number during eye development. Reduction of cell number can occur through either increased cell death, or decreased cell proliferation (Kramer & Staveley, 2003).

Examination of both reduced and increased levels of *CG34126* in the *Drosophila* eye were carried out using the transgenic eye tissue-specific *GMR-Gal4*, and the characteristics were analysed to identify the effects of altered gene expression. Directed overexpression and inhibition of *UHRF1BP1L* homologues in the eye showed no significant difference from the control, when compared for ommatidia or bristle numbers. Inhibition or overexpression of *CG34126* did not alter the normal development of the eye and seems to have a limited role in neurodevelopment under normal cellular conditions or other factors contribute to this counterbalancing effect. In a recent study that introduced a number of new PD candidate genes (Jansen *et al.,* 2017), α-synuclein-induced retinal degeneration and screening assay in *Drosophila*; they did not observe significant retinal pathology in α-synuclein null flies directed by Rhodopsin1-GAL4 but when the transgene was induced by α-synuclein it showed retinal degeneration with synergistic effects. It did not show any neuro development in the eyes of *CG34126* regulated fly, but experiment with derivative flies or α-synuclein induced flies might show the expected PD phenotypes.

Patient of PD commonly suffered from early death as well as other PD symptoms (Forno, 1996). In these studies, I have detected that the inhibition of *CG34126* of via RNA-interference in flies directed by *D42-Gal4, Ddc-Gal4* and *TH-Gal4* have a deleterious effect on the survival rate compared to control, which is characteristic of PD-like phenotype. Same type of results has been observed in previous ageing analysis of flies, where due to the loss-of-function of *park*, flies showed shortened lifespan alongside reduced climbing ability and degeneration of DA neurons compared to the control (Greene

et al., 2003; Whitworth *et al.,* 2005). Similarly, a decrease in *nutcracker/Fbxo7* expression directed by *Ddc-Gal4* caused a significant reduction in mean lifespan of flies (Merzetti *et al.,* 2016). It may be possible that suppression of *CG34126* creates inhibition in the normal function of the dopaminergic and motor neurons, and as a result the experimental flies died earlier than the control. However, the inhibition line *UAS-CG34126-RNAi^{KK}* did not demonstrate any significant changes to the lifespan. Overexpression of *CG34126* had no significant role in the ageing process except for the flies expressed under 3,4-dihydroxyphenylalanine (DOPA) decarboxylase gene promoter *Ddc-GAL4*, which have a shorter lifespan compare to the control. Any previous experiment has not been carried out in a longevity assay associated with *UHRF1BP1L* homologue in flies, therefore the exact reason for this reduction in lifespan due to inhibition and overexpression of *CG34126* is unclear. An increase in programmed cell death or a decrease in cell growth during development, coupled with selective apoptotic death of these DA neurons due to altered expression of CG34126, may be the contributing factor which ultimately decreased cellular protection and survival of the cell and thereby decreased survival rate of the fly. Dopaminergic neurons may die as a result of apoptosis in PD (Lev *et al.,* 2003). Exploration of the role of cell survival signalling in the selective loss of dopaminergic neurons in *Drosophila* may provide further insight into the molecular basis of PD.

Locomotor disorder is one of the dopaminergic neuron-associated characteristics of PD. As the transgenic flies recapitulate some of the prominent features of PD, such as the degeneration of DA neurons and the age-dependent loss of locomotor capability,

movement analysis was conducted to determine the effect of *CG34126* on climbing ability of *Drosophila* over time. Climbing ability acts as a key indicator of phenotypic expression of a particular gene in DA neurons. Analysis of climbing to determine the phenotypic effects of overexpression and inhibition of genes, especially to recuperate the α-synuclein induced PD phenotypes have been conducted many times (Feany & Bender, 2000; Haywood & Staveley, 2006; Todd & Staveley, 2008). Aucluck *at el.,* (2002), used the *UAS-GAL4* system to regulate transgenic expression of the Chaperone Hsp 70 using the *Ddc-Gal4* line in flies and found that direct expression of the Hsp70 mitigated dopaminergic neuronal loss related with α-synuclein in flies and inhibition of Hsp70 promoting α-synuclein induced neurodegeneration. In another transgenic experiment, it has been speculated that loss-of-function of *Drosophila* orthologue of human *LRRK* led to severe locomotor abnormality (Lee *et al*., 2007). In my experiment, I observed that suppression of both inhibition lines of *CG34126* in flies with the three gene promoter *Ddc- Gal4*, *D42-Gal4,* and *TH-Gal4* caused a noticeable reduction in locomotor ability over time compared to control flies. Similar observations have been noticed by Feany & Bender, (2000), where the flies were unable to move upward beyond the first section of the climbing apparatus at the very end of their lives. *CG34126* is producing similar effects in *Drosophila* as to what would be expected by knockdown of *UHRF1BP1L*. This may be because loss-of-function of *CG34126* negatively affects the dopaminergic neurons' surveillance and defects in mitochondrial biogenesis by interfering protein transport. There were no changes in climbing ability for the overexpression of *CG34126* in flies except in those, who were controlled under the *Ddc-Gal4* transgene, which showed a decreased in movement ability

with progression of time. It is apparent that one inhibition line has more severe ageing effects compare to another inhibition line, but both lines represented decline in the climbing ability over time. The inhibition of *CG34126* under the control of DA and motor neurons result in short lifespan and impaired locomotor function, phenotypes that are strongly relatable to the *Drosophila* models of PD. Flies have phenotypes that are consistent modelling PD in *Drosophila* through alteration of *CG34126*.

Parkin takes part in protein ubiquitination through affecting the function of the ubiquitin-ligase enzyme, and mutation of it is involved in abnormal protein aggregation causes decease of neuronal cell (Shimura *et al*., 2000). Mutual interaction of *PINK1* and *parkin* maintains the mitochondrial integrity. Overexpression of mitochondrial fission regulating gene in *Drosophila* rescues the phenotypes of muscular defects and mitochondrial dysfunction in *park* mutant flies (Deng *et al.,* 2008). In our study, co-expression of *park-RNAi* and *CG34126* overexpression under the control of dopaminergic *Ddc-Gal4*, resulted in an improved climbing ability compared to the control. Moreover, overexpression of *CG34126* positively acted to retrieve survival ability in *park* RNA-interference flies. Similarly, the inhibition of *porin* along with the overexpression of *Buffy* in the DA neurons results in the suppression of the age-dependent loss in climbing ability and survival rate (M'Angale & Staveley, 2016b). In another experiment, it was found that overexpression of *Pink1* rescued the premature loss of climbing abilities induced by *α-synuclein* (Todd and Staveley, 2008). In this experiment when *CG34126* was overexpressed in dopaminergic neurons, the flies lived and climbed longer than the control. It recuperates both climbing and survival ability of park RNA-interference flies. It can be

predicted that overexpression of *CG34126* may have positive role in maintaining mitochondrial dynamics, cell growth, and overcome the toxicity produced by *park* suppression. On the other hand, co-inhibition of both genes *park-RNAi* and *UAS-CG34126-RNAi*^{GD} in DA neurons under the dopaminergic neuron-specific transgene deteriorated not only the movement ability but also the lifespan of transgenic flies. Another inhibition line *UAS-CG34126-RNAi*^{KK} showed normal lifespan with decreased locomotor power when crossed with *park-RNAi* regulated by same *Ddc-Gal4* transgene. Inhibition of both gene *CG34126* and *park* in the flies demonstrated a significant reduction in climbing and survival ability and indicated that reduced level of *park* and *CG34126* expression might have detrimental effects, which is relatable to the symptoms of PD affected patients.

Conclusion

Characterization of the *Drosophila melanogaster* homologue of *UHFR1BP1L* has been done through the overexpression and inhibition of the *CG34126* gene in eye development, longevity and climbing ability along with bioinformatic analysis. UHRF1BP1L protein is well conserved in functional protein trafficking domains of both human and *Drosophila*. However, this protein is not highly conserved at the amino acid level due to the evolutionary genetic divergence between vertebrates and invertebrates but conserved in their respective groups. Loss-of-function of *CG34126* leads to reduced longevity with climbing and gives a model of PD. Gain-of-function does not provide any prominent PD symptoms but it rescues the *parkin* RNAi model of PD by increasing both longevity and locomotor ability. Further cellular and molecular study and interaction of *UHRF1BP1L* and its homologue *CG34126* with others PD genes such as *α-synuclein*, *PINK1*, and *VPS13C* can be carried out for the manipulation of drugs that possibly function better in the PD affected patients.

References

Abou-Sleiman, P.M., Muqit, M.M., & Wood, N.W., (2006). Expanding insights of mitochondrial dysfunction in Parkinson's disease. *Nature Reviews Neuroscience,* 7(3): 207-219.

Aharon-Peretz, J., Rosenbaum, H., & Gershoni-Baruch, R., (2004). Mutations in the glucocerebrosidase gene and Parkinson's disease in Ashkenazi Jews. *New England Journal of Medicine,* 351(19): 1972-1977.

Ashrafi, G., Schlehe, J.S., LaVoie, M.J., & Schwarz, T.L., (2014). Mitophagy of damaged mitochondria occurs locally in distal neuronal axons and requires PINK1 and Parkin. *Journal of Cell Biology,* 206(5): 655-670.

Atkin, G., & Paulson, H., (2014). Ubiquitin pathways in neurodegenerative disease. *Frontiers in Molecular Neuroscience, 7*: 63.

Auluck, P.K., Chan, H.Y., Trojanowski, J.Q., Lee, V.M., & Bonini, N.M., (2002). Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for Parkinson's disease. *Science,* 295(5556): 865-868.

Auluck, P.K., Meulener, M.C., & Bonini, N.M., (2005). Mechanisms of Suppression of alpha-Synuclein Neurotoxicity by Geldanamycin in Drosophila. *Journal of Biological Chemistry,* 280(4): 2873-2878.

Baker, N.E., (2001). Cell proliferation, survival, and death in the Drosophila eye. *Seminars in Cell & Developmental Biology,* 12(6): 499-507.

Banerjee, R., Starkov, A.A., Beal, M.F., & Thomas, B., (2009). Mitochondrial dysfunction in the limelight of Parkinson's disease pathogenesis. *Biochimica et Biophysica Acta (BBA)-Molecular Basis Disease,* 1792(7): 651-663.

Barone, P., (2010). Neurotransmission in Parkinson's disease: beyond dopamine. *European Journal of Neurology,* 17(3): 364-376.

Beckingham, K.M., Armstrong, J.D., Texada, M.J., Munjaal, R., & Baker, D.A., (2007). *Drosophila melanogaster*--the model organism of choice for the complex biology of multi-cellular organisms. *Journal of the American Society for Gravitational and Space Biology,* 18(2):17-29.

Bellen, H.J., Levis, R.W., He, Y., Carlson, J.W., Evans-Holm, M., Bae, E., Kim, J., Metaxakis, A., Savakis, C., Schulze, K.L., Hoskins, R.A., & Spradling, A.C., (2011). The Drosophila gene disruption project: progress using transposons with distinctive site specificities. *Genetics,* 188(3): 731-743.

Bexiga, M.G., & Simpson, J.C., (2013). Human diseases associated with form and function of the Golgi complex. *International Journal of Molecular Sciences,* 14(9): 18670-18681.

Bonifati, V., Rizzu, P., Squitieri, F., Krieger, E., Vanacore, N., van Swieten, J.C., Brice, A., Van Duijn, C.M., Oostra, B., Meco, G., & Heutink, P., (2003). DJ-1 (PARK7), a novel gene for autosomal recessive, early onset parkinsonism. *Neurological Sciences,* 24(3): 159-160.

Borland, M.K., Trimmer, P.A., Rubinstein, J.D., Keeney, P.M., Mohanakumar, K., Liu, L., & Bennett, J. P. Jr, (2008). Chronic, low-dose rotenone reproduces lewy neurites found in early stages of Parkinson's disease, reduces mitochondrial movement and slowly kills differentiated SH-SY5Y neural cells. *Molecular Neurodegeneration,* 3: 21.

Bose, A., & Beal, M.F., (2016). Mitochondrial dysfunction in Parkinson's disease. *Journal of Neurochemistry,* 139(Suppl. 1): 216-231.

Botella, J.A., Bayersdorfer, F., Gmeiner, F., & Schneuwly, S., (2009). Modelling Parkinson’s disease in Drosophila. *Neuromolecular Medicine,* 11(4): 268-280.

Boto, T., Louis, T., Jindachomthong, K., Jalink, K., & Tomchik, S.M., (2014). Dopaminergic modulation of cAMP drives nonlinear plasticity across the Drosophila mushroom body lobes. *Current Biology,* 24(8): 822-831.

Brand, A.H., Manoukian, A.S., & Perrimon, N., (1994). Ectopic expression in Drosophila. *Methods in Cell Biology* 44: 635-654.

Brandhorst, D., Zwilling, D., Rizzoli, S. O., Lippert, U., Lang, T., & Jahn, R., (2006). Homotypic fusion of early endosomes: SNAREs do not determine fusion specificity. *Proceedings of the National Academy of Sciences of the United States of America,* 103(8): 2701-2706.

Burdett, H., & van den Heuvel, M., (2004). Fruits and flies: a genomics perspective of an invertebrate model organism. *Briefings in Functional Genomics and Proteomics,* 3(3): 257-266.

Bronner, C., Achour, M., Arima, Y., Chataigneau, T., Saya, H., & Schini-Kerth, V. B. (2007). The UHRF family: oncogenes that are drugable targets for cancer therapy in the near future. *Pharmacology & therapeutics*.115(3):419-434.

Cavallarin, N., Vicario, M., & Negro, A., (2010). The role of phosphorylation in synucleinopathies: Focus on Parkinson's disease. *CNS & Neurological Disorders-Drug Targets,* 9(4): 471-481.

Chan, N.C., Salazar, A.M., Pham, A.H., Sweredoski, M.J., Kolawa, N.J., Graham, R.L., Graham, R.L., Hess, S., & Chan D.C., (2011). Broad activation of the ubiquitin-proteasome system by Parkin is critical for mitophagy. *Human Molecular Genetics,* 20(9): 1726-1737.

Chartier-Harlin, M., Dachsel, J. C., Vilariño-Güell, C., Lincoln, S.J., Leprêtre, F., Hulihan, M.M., Kachergus, J., Milnerwood, A.J., Tapia, L., Song, M.S., Le Rhun E., Mutez, E., Larvor, L., Duflot, A., Vanbesien-Mailliot, C., Kreisler, A., Ross, O.A., Nishioka, K., Soto-Ortolaza, A.I., Cobb, S.A., Melrose, H.L., Behrouz, B., Keeling, B.H., Bacon, J.A., Hentati, E., Williams, L., Yanagiya, A., Sonenberg, N., Lockhart, P.J., Zubair, A.C., Uitti, R.J., Aasly, J.O., Krygowska-Wajs, A., Opala, G., Wszolek, Z.K., Frigerio, R., Maraganore, D.M., Gosal, D., Lynch, T., Hutchinson, M., Bentivoglio, A.R., Valente, E.M., Nichols, W.C., Pankratz, N., Foroud, T., Gibson, R.A., Hentati, F., Dickson, D.W., Destée, A., & Farrer, M.J., (2011). Translation initiator EIF4G1 mutations in familial Parkinson disease. *American Journal of Human Genetics,* 89(3): 398-406.

Chaturvedi, R.K., & Beal, M.F., (2008). Mitochondrial approaches for neuroprotection. *Annals of the New York Academy of Sciences,* 1147(1): 395-412.

Chesi, A., Kilaru, A., Fang, X., Cooper, A.A., & Gitler, A.D., (2012). The role of the Parkinson's disease gene PARK9 in essential cellular pathways and the manganese homeostasis network in yeast. *PloS One,* 7(3): e34178.

Chiang, A., Lin, C., Chuang, C., Chang, H., Hsieh, C., Yeh, C., Shih, C.T., Wu, J.J., Wang, G.T., Chen, Y.C., Wu, C.C., Chen, G.Y., Ching, Y.T., Lee, P.C., Lin, C.Y., Lin, H.H., Wu, C.C., Hsu, H.W., Huang, Y.A., Chen, J.Y., Chiang, H.J., Lu, C.F., Ni, R.F., Yeh, C.Y., & Hwang, J.K., (2011). Three-dimensional reconstruction of brain-wide wiring networks in Drosophila at single-cell resolution. *Current Biology,* 21(1): 1-11.

Chintapalli, V.R., Wang, J., & Dow, J.A., (2007). Using FlyAtlas to identify better Drosophila melanogaster models of human disease. *Nature Genetics,* 39(6): 715-720.

Chung, C.Y., Khurana, V., Auluck, P.K., Tardiff, D. F., Mazzulli, J.R., Soldner, F., Baru V., Lou Y., Freyzon Y., Cho S., Mungenast A.E., Muffat, J., Mitalipova, M., Pluth, M.D., Jui, N.T., Schüle, B., Lippard, S.J., Tsai, L.H., Krainc, D., Buchwald, S.L., Jaenisch, R,, & Lindquist, S., (2013). Identification and rescue of α-synuclein toxicity in Parkinson patient-derived neurons. *Science,* 342(6161): 983-987.

Clarimón, J., Eerola, J., Hellström, O., Peuralinna, T., Tienari, P.J., & Singleton, A.B., (2006). Assessment of PINK1 (PARK6) polymorphisms in finnish PD. *Neurobiology of Aging,* 27(6): 906-907.

Clark, I.E., Dodson, M.W., Jiang, C., Cao, J.H., Huh, J.R., Seol, J.H., Yoo, S.J., Hay, B.A., & Guo, M., (2006). Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature,* 441(7097): 1162-1166.

Cookson, M.R., (2012). Parkinsonism due to mutations in PINK1, parkin, and DJ-1 and oxidative stress and mitochondrial pathways. *Cold Spring Harbor Perspectives in Medicine,* 2(9): a009415.

Cooper, A.A., Gitler, A.D., Cashikar, A., Haynes, C.M., Hill, K.J., Bhullar B, Liu K., Xu K., Strathearn K., Liu F., Cao, S., Caldwell, K.A., Caldwell, G.A., Marsischky, G., Kolodner, R.D., Labaer, J., Rochet, J.C., Bonini, N.M., & Lindquist, S., (2006).

Alpha-synuclein blocks ER-golgi traffic and Rab1 rescues neuron loss in Parkinson's models. *Science,* 313(5785): 324-328.

Cording, A.C., Shiaelis, N., Petridi, S., Middleton, C.A., Wilson, L.G., & Elliott, C.J., (2017). Targeted kinase inhibition relieves slowness and tremor in a Drosophila model of LRRK2 parkinson's disease. *NPJ Parkinson's disease,* 3(1): 34.

Credle, J.J., Forcelli, P.A., Delannoy, M., Oaks, A.W., Permaul, E., Berry, D.L., Duka V., Wills J., & Sidhu A., (2015). α-Synuclein-mediated inhibition of ATF6 processing into COPII vesicles disrupts UPR signaling in Parkinson's disease. *Neurobiology of Disease,* 76: 112-125.

da Costa, C.A., (2007). DJ-1: a new comer in Parkinson's disease pathology. *Current Molecular Medicine,* 7(7): 650-657.

Daher, J.P., Abdelmotilib, H.A., Hu, X., Volpicelli-Daley, L.A., Moehle, M.S., Fraser, K. B., Needle, E., Chen, Y., Steyn, S., Galatsis, P., Hirst, W.D., & West, A.B., (2015). Leucine-rich repeat kinase 2 (LRRK2) Pharmacological Inhibition Abates α-Synuclein Gene-induced Neurodegeneration. *Journal of Biological Chemistry,* 290(32): 19433-19444.

Dantuma, N.P., & Bott, L.C., (2014). The ubiquitin-proteasome system in neurodegenerative diseases: precipitating factor, yet part of the solution. *Frontiers in Molecular Neuroscience,* 7: 70.

Dawson, T.M., & Dawson, V.L., (2003). Molecular pathways of neurodegeneration in Parkinson's disease. *Science*, 302(5646): 819-822.

Deas, E., Cremades, N., Angelova, P.R., Ludtmann, M. H., Yao, Z., Chen, S., Horrocks M.H., Banushi, B., Little, D., Devine, M.J., Gissen, P., Klenerman, D., Dobson, C.M., Wood, N.W., Gandhi, S., & Abramov, A.Y., (2016). Alpha-synuclein Oligomers Interact with Metal Ions to Induce Oxidative Stress and Neuronal Death in Parkinson's disease. *Antioxidants & Redox Signaling,* 24(7): 376-391.

Deng, H., Dodson, M.W., Huang, H., & Guo, M., (2008). The Parkinson's disease genes pink1 and parkin promote mitochondrial fission and/or inhibit fusion in Drosophila. *Proceedings of the National Academy of Sciences of the United States of America,* 105(38): 14503-14508.

Derbyshire, M.K., Gonzales, N.R., Lu, S., He, J., Marchler, G.H., Wang, Z., & Marchler-Bauer A., (2015). Improving the consistency of domain annotation within the Conserved Domain Database. *Database, Journal of Biological Batabases and curation,* 2015.

Di Fonzo, A., Dekker, M. C., Montagna, P., Baruzzi, A., Yonova, E. H., Correia Guedes, L., Szczerbinska A., Zhao T., Dubbel, L., Wouters, C.,de Graaff, E., Oyen, W.J.G., Simons, J.E., Breedveld, G.J., Oostra, B.A., Horstink, M.W., & Bonifati, V., (2009). FBXO7 mutations cause autosomal recessive, early-onset parkinsonian-pyramidal syndrome. *Neurology,* 72(3), 240-245.

Dobzhansky, T., & Wallace, B., (1953). The genetics of homeostasis in Drosophila. *Proceedings of the National Academy of Sciences of the United States of America,* 39(3), 162-171.

Dorsey, E.R., Constantinescu, R., Thompson, J.P., Biglan, K.M., Holloway, R.G., Kieburtz, K., Marshall, F.J., Ravina, B.M., Schifitto, G., Siderowf, A., & Tanner, C.M., (2007). Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology,* 68(5): 384-386.

Drapalo, K., & Jozwiak, J., (2017). Parkin, PINK1 and DJ1 as possible modulators of mTOR pathway in ganglioglioma. *International Journal of Neuroscience,* 128(2):176-174.

Ebrahimi-Fakhari, D., Wahlster, L., & McLean, P.J., (2012). Protein degradation pathways in Parkinson's disease: Curse or blessing. *Acta Neuropathologica,* 124(2): 153-172.

Edvardson, S., Cinnamon, Y., Ta-Shma, A., Shaag, A., Yim, Y., Zenvirt, S., Jalas, C., Lesage, S., Brice, A., Taraboulos, A., Kaestner, K.H., Greene, L.E., & Elpeleg, O., (2012). A deleterious mutation in DNAJC6 encoding the neuronal-specific clathrin-uncoating co-chaperone auxilin, is associated with juvenile parkinsonism. *PloS One,* 7(5): e36458.

Eijkelenboom, A., & Burgering, B.M.T., (2013). FOXOs: Signalling integrators for homeostasis maintenance. *Nature Reviews, Molecular Cell Biology,* 14(2): 83-97.

Eriksen, J.L., Wszolek, Z., & Petrucelli, L., (2005). Molecular pathogenesis of Parkinson disease. *Archives of Neurology,* 62(3): 353-357.

Exner, N., Lutz, A.K., Haass, C., & Winklhofer, K.F., (2012). Mitochondrial dysfunction in Parkinson's disease: Molecular mechanisms and pathophysiological consequences. *The EMBO Journal, 31*(14): 3038-3062.

Fanali, G., Rampoldi, V., Masi, A. D., Bolli, A., Lopiano, L., Ascenzi, P., & Fasano, M., (2010). Binding of anti-Parkinson's disease drugs to human serum albumin is allosterically modulated. *Journal of the International Union of Biochemistry and Molecular Biology Life,* 62(5): 371-376.

Feany, M.B., & Bender, W.W., (2000). A Drosophila model of Parkinson's disease. *Nature, 404*(6776): 394-398.

Finney, N., Walther, F., Mantel, P.Y., Stauffer, D., Rovelli, G., & Dev, K.K., (2003). The cellular protein level of parkin is regulated by its ubiquitin-like domain. *Journal of Biological Chemistry,* 278(18): 16054-16058.

Follett, J., Norwood, S.J., Hamilton, N.., Mohan, M., Kovtun, O., Tay, S., Zhe Y, Wood, S.A., Mellick, G.D., Silburn, P.A., Collins, B.M., Bugarcic, A., & Teasdale, R.D., (2014). The Vps35 D620N mutation linked to Parkinson's disease disrupts the cargo sorting function of retromer. *Traffic,* 15(2): 230-244.

Forno, L.S., (1996). Neuropathology of Parkinson's disease. *Journal of Neuropathology and Experimental Neurology, 55*(3): 259-272.

Franz, A., Kevei, E., & Hoppe, T., (2015). Double-edged alliance: Mitochondrial surveillance by the UPS and autophagy. *Current Opinion in Cell Bbology, 37*: 18-27.

Freeman, M., (1996). Reiterative use of the EGF receptor triggers differentiation of all cell types in the Drosophila eye. *Cell,* 87(4): 651-660.

Friggi-Grelin, F., Coulom, H., Meller, M., Gomez, D., Hirsh, J., & Birman, S., (2003). Targeted gene expression in Drosophila dopaminergic cells using regulatory sequences from tyrosine hydroxylase. *Journal of Neurobiology,* 54(4): 618-627.

Gandhi, S., Muqit, M., Stanyer, L., Healy, D., Abou-Sleiman, P., Hargreaves, I., Heales, S., Ganguly, M., Parsons, L., Lees, A.J., Latchman, D.S., Holton, J.L., Wood, N.W., & Revesz, T., (2006). PINK1 protein in normal human brain and Parkinson's disease. *Brain,* 129(7): 1720-1731.

Geer, L.Y., Domrachev, M., Lipman, D.J., & Bryant, S.H., (2002). CDART: Protein homologuey by domain architecture. *Genome Research,* 12(10): 1619-1623.

Greene, J.C., Whitworth, A.J., Kuo, I., Andrews, L.A., Feany, M.B., & Pallanck, L.J., (2003). Mitochondrial pathology and apoptotic muscle degeneration in Drosophila parkin mutants. *Proceedings of the National Academy of Sciences of the United States of America,* 100(7): 4078-4083.

Haelterman, N.A., Yoon, W.H., Sandoval, H., Jaiswal, M., Shulman, J.M., & Bellen, H.J., (2014). A mitocentric view of Parkinson's disease. *Annual Review of Neuroscience,* 37: 137-159.

Hattori, N., & Mizuno, Y., (2004). Pathogenetic mechanisms of parkin in Parkinson's disease. *Lancet,* 364(9435): 722-724.

Hawthorne, F., Feng, S., Metlapally, R., Li, Y., Tran-Viet, K., Guggenheim, J.A., Malecaze, F., Calvas, P., Rosenberg, T., Mackey, D.A., Venturini, C., Hysi, P.G., Hammond, J.C. & Young, T.L., (2013). Association mapping of the high-grade myopia MYP3 locus reveals novel candidates UHRF1BP1L, PTPRR, and PPFIA2Mapping of the high-grade myopia MYP3 locus. *Investigative Ophthalmology & Visual Science,* 54(3): 2076-2086.

Hayashi, T., Kishida, M., Nishizawa, Y., Iijima, M., Koriyama, C., Nakamura, M., Sano, A., & Kishida, S., (2012). Subcellular localization and putative role of VPS13A/chorein in dopaminergic neuronal cells. *Biochemical and Biophysical Research Communications,* 419(3); 511-516.

Haywood, A.F., & Staveley, B.E., (2006). Mutant alpha-synuclein-induced degeneration is reduced by parkin in a fly model of Parkinson's disease. *Genome,* 49(5): 505-510.

Henchcliffe, C., & Beal, M.F., (2008). Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis. *Nature Clinical Practice Neurology,* 4(11): 600-609.

Heo, J., & Rutter, J., (2011). Ubiquitin-dependent mitochondrial protein degradation. *International Journal of Biochemistry & Cell Biology,* 43(10): 1422-1426.

Hochstrasser, M., (1996). Ubiquitin-dependent protein degradation. *Annual Review of Genetics,* 30(1): 405-439.

Hornykiewicz, O., (1962). Dopamine (3-hydroxytyramine) in the central nervous system and its relation to the Parkinson syndrome in man. *Deutsche Medizinische Wochenschrift, 87*: 1807-1810.

Hu, Y., Dammer, E.B., Ren, R., & Wang, G., (2015). The endosomal-lysosomal system: from acidification and cargo sorting to neurodegeneration. *Translational Neurodegeneration,* 4: 18.

Imai, Y., Soda, M., Inoue, H., Hattori, N., Mizuno, Y., & Takahashi, R., (2001). An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of Parkin. *Cell,* 105(7): 891-902.

Ishikawa, K., Nagase, T., Suyama, M., Miyajima, N., Tanaka, A., Kotani, H., Nomura, N., & Ohara, O., (1998). Prediction of the coding sequences of unidentified human genes. X. the complete sequences of 100 new cDNA clones from brain which can code for large proteins in vitro. *DNA Research,* 5(3): 169-176.

Jacobs, S., & O'neil, K., (2018). Fibronectin Type III Domain Based Scaffold Compositions, Methods and Uses, U.S. Patent Application No. 10/040,842.

Jansen, I.E., Ye, H., Heetveld, S., Lechler, M.C., Michels, H., Seinstra, R.I., Lubbe, S.J., Drouet, V., Lesage, S., Majounie, E., Gibbs, J.R., Nalls, M.A., Ryten, M., Botia, J.A., Vandrovcova, J., Simon-Sanchez, J., Castillo-Lizardo, M., Rizzu, P., Blauwendraat, C., Chouhan, A.K., Li, Y., Yogi, P., Amin, N., van Duijn, C.M., Morris, H.R., Brice, A., Singleton, A.B., David, D.C., Nollen, E.A., Jain, S., Shulman, J.M., & Heutink, P., (2017). Discovery and functional prioritization of Parkinson’s disease candidate genes from large-scale whole exome sequencing. *Genome Biology,* 18(1): 22.

Jensen, K.L., Runegaard, A.H., Weikop, P., Gether, U., & Rickhag, M., (2017). Assessment of Dopaminergic Homeostasis in Mice by Use of High-Performance Liquid Chromatography Analysis and Synaptosomal Dopamine Uptake. *Journal of Visualized Experiments,* 127: 56093.

Kalinderi, K., Bostantjopoulou, S., & Fidani, L., (2016). The genetic background of parkinson's disease: current progress and future prospects. *Acta Neurologica Scandinavica,* 134(5): 314-326.

Kamp, F., Exner, N., Lutz, A.K., Wender, N., Hegermann, J., Brunner, B., Nuscher, B., Bartels, T., Giese, A., Beyer, K., Eimer, S., Winklhofer, K.F., & Haass C., (2010). Inhibition of mitochondrial fusion by α-synuclein is rescued by PINK1, Parkin and DJ-1. *European Molecular Biology Organization*, *29*(20), 3571-3589.

Kane, L.A., Lazarou, M., Fogel, A.I., Li, Y., Yamano, K., Sarraf, S.A., Banerjee, S., & Youle, R.J., (2014). PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. *Journal of Cell Biology,* 205(2): 143-153.

Kazlauskaite, A., Kondapalli, C., Gourlay, R., Campbell, D.G., Ritorto, M.S., Hofmann, K., Hofmann, K., Alessi, D.R., Knebel, A., Trost, M., & Muqit, M.M., (2014). Parkin is activated by PINK1-dependent phosphorylation of ubiquitin at Ser65. *Biochemical Journal,* 460(1): 127-139.

Kim, C.Y., & Alcalay, R.N., (2017). Genetic forms of Parkinson's disease. *Seminars in Neurology, ,* 37(2): 135-146.

Kim, R.H., Peters, M., Jang, Y., Shi, W., Pintilie, M., Fletcher, G.C., DeLuca, C., Liepa, J., Zhou, L., Snow, B., Binari, R.C., Manoukian, A.S., Bray, M.R., Liu, F.F., Tsao, M.S., & Mak,T.W., (2005). DJ-1, a novel regulator of the tumor suppressor PTEN. *Cancer Cell,* 7(3): 263-273.

Kinghorn, K.J., Castillo-Quan, J.I., Bartolome, F., Angelova, P.R., Li, L., Pope, S., Cocheme, H.M., Khan, S., Asghari, S., Bhatia, K.P., Hardy J., Abramov, A.Y., & Patridge, L., (2015). Loss of PLA2G6 leads to elevated mitochondrial lipid peroxidation and mitochondrial dysfunction. *Brain,* 138(7): 1801-1816.

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y., & Shimizu, N., (1998). Mutations in the parkin gene cause autosomal recessive juvenile Parkinsonism. *Nature,* 392(6676): 605-608.

Kramer, J.M., & Staveley, B.E., (2003). GAL4 causes developmental defects and apoptosis when expressed in the developing eye of Drosophila melanogaster. *Genetics and Molecular Research,* 2(1): 43-47.

Krüger, R., (2004). Genes in familial parkinsonism and their role in sporadic Parkinson's disease. *Journal of Neurology,* 251(2): 2-6.

Kumar, A., Christian, P.K., Panchal, K., Guruprasad, B., & Tiwari, A.K., (2017). Supplementation of Spirulina (Arthrospira platensis) Improves Lifespan and Locomotor Activity in Paraquat-Sensitive DJ-1$\beta^{\Delta 93}$ Flies, a Parkinson's Disease Model in Drosophila melanogaster. *Journal of Dietary Supplements,* 14(5): 573-588.

Kumar, J.P., (2012). Building an ommatidium one cell at a time. *Developmental Dynamics,* 241(1): 136-149.

Kumar, N., Leonzino, M., Hancock-Cerutti, W., Horenkamp, F. A., Li, P., Lees, J.A., Wheeler, H., Reinisch, K.M., & De Camilli, P., (2018). VPS13A and VPS13C are lipid transport proteins differentially localized at ER contact sites. *Journal of Cell Biology,* 217(10): 3625-3639.

Lebouvier, T., Neunlist, M., des Varannes, S.B., Coron, E., Drouard, A., N'Guyen, J., Chaumette, T., Tasselli, M., Paillusson, S., Flamand, M., & Galmiche, J.P., (2010). Colonic biopsies to assess the neuropathology of Parkinson's disease and its relationship with symptoms. *PloS One,* 5(9): e12728.

Lee, V.M., Goedert, M., & Trojanowski, J.Q., (2001). Neurodegenerative tauopathies. *Annual Review of Neuroscience,* 24(1): 1121-1159.

Lee, S.B., Kim, W., Lee, S., & Chung, J., (2007). Loss of LRRK2/PARK8 induces degeneration of dopaminergic neurons in Drosophila. *Biochemical and Biophysical Research Communications*, 358(2): 534-539.

Leroy, E., Boyer, R., Auburger, G., Leube, B., Ulm, G., Mezey, E., Harta G, Brownstein, M.J., Jonnalagada, S., Chernova,T., Dehejia, A., Lavedan, C., Gasser, T., Steinbach, P.J., Wilkinson, K.D., & Polymeropoulos, M.H., (1998). The ubiquitin pathway in Parkinson's disease. *Nature,* 395(6701): 451-452.

Lesage, S., & Brice, A., (2009). Parkinson's disease: from monogenic forms to genetic susceptibility factors. *Human Molecular Genetics,* 18(1): 48-59.

Lesage, S., Drouet, V., Majounie, E., Deramecourt, V., Jacoupy, M., Nicolas, A., Cormier-Dequaire, F., Hassoun, S.M., Pujol, C., Ciura, S., Erpapazoglou, Z., Usenko, T., Maurage, C.A., Sahbatou, M., Liebau, S., Ding, J., Bilgic, B., Emre, M., Erginel-Unaltuna, N., Guven, G., Tison, F., Tranchant, C., Vidailhet, M., Corvol, J.C., Krack, P., Leutenegger, A.L., Nalls, M.A., Hernandez, D.G., Heutink, P., Gibbs, J.R., Hardy, J., Wood, N.W., Gasser, T., Durr, A., Deleuze, J.F., Tazir, M., Destee, A., Lohmann, E., Kabashi, E., Singleton, A., Corti, O., & Brice A. (2016). Loss of VPS13C Function in Autosomal-Recessive Parkinsonism Causes Mitochondrial Dysfunction and

Increases PINK1/Parkin-Dependent Mitophagy. *American Journal of Human Genetics,* 98(3): 500-513.

Lev, N., Melamed, E., & Offen, D., (2003). Apoptosis and Parkinson's disease. *Progress in Neuro-Psychopharmacology and Biological Psychiatry,* 27(2): 245-250.

Lev, N., Roncevich, D., Ickowicz, D., Melamed, E., & Offen, D., (2006). Role of DJ-1 in Parkinson's disease. *Journal of Molecular Neuroscience,* 29(3): 215-225.

Li, B., Hu, Q., Wang, H., Man, N., Ren, H., Wen, L., Nukina, N., Fei, E., & Wang, G., (2010). Omi/HtrA2 is a positive regulator of autophagy that facilitates the degradation of mutant proteins involved in neurodegenerative diseases. *Cell Death and Differentiation,* 17(11): 1773-1784.

Li, H., Chaney, S., Forte, M., & Hirsh, J., (2000). Ectopic G-protein expression in dopamine and serotonin neurons blocks cocaine sensitization in Drosophila melanogaster. *Current Biology,* 10(4): 211-214.

Liu, Z., Wang, X., Yu, Y., Li, X., Wang, T., Jiang, H., Ren, Q., Jiao, Y., Sawa, A., Moran,T., & Ross, C.A., (2008). A Drosophila model for LRRK2-linked Parkinsonism. *Proceedings of the National Academy of Sciences of the United States of America,* 105(7): 2693-2698.

M’Angale, P. G., & Staveley, B. E., (2016a). Loss of porin function in dopaminergic neurons of Drosophila is suppressed by buffy. *Journal of Biomedical Science,* 23(1): 84.

M’Angale, P. G., & Staveley, B. E., (2016b). Overexpression of buffy enhances the loss of parkin and suppresses the loss of Pink1 phenotypes in Drosophila. *Genome,* 60(3): 241-247.

MacLeod, D.A., Rhinn, H., Kuwahara, T., Zolin, A., Di Paolo, G., McCabe, B. D., Marder, K.S., Honig, L.S., Clark, L.N., Small, S.A., & Abeliovich, A., (2013). RAB7L1 interacts with LRRK2 to modify intraneuronal protein sorting and Parkinson’s disease risk. *Neuron,* 77(3): 425-439.

Marsh, J.L., Pallos, J., & Thompson, L.M., (2003). Fly models of Huntington's disease. *Human Molecular Genetics,* 12(2): 187-:193.

Martin, I., Kim, J.W., Dawson, V.L., & Dawson, T.M., (2014). LRRK2 pathobiology in Parkinson's disease. *Journal of Neurochemistry,* 131(5): 554-565.

Menzies, F.M., Yenisetti, S.C., & Min, K., (2005). Roles of Drosophila DJ-1 in survival of dopaminergic neurons and oxidative stress. *Current Biology,* 15(17): 1578-1582.

Mercado, G., Castillo, V., Soto, P., & Sidhu, A., (2016). ER stress and Parkinson's disease: Pathological inputs that converge into the secretory pathway. *Brain Research,* 1648: 626-632.

Merzetti, E.M., & Staveley, B.E., (2016). Identifying potential PARIS homologues in D. melanogaster. *Genetics and Molecular Research,* 15(4): gmr15048934.

Merzetti, E.M., Dolomount, L.A., & Staveley, B.E., (2016). The FBXO7 homologue nutcracker and binding partner PI31 in Drosophila melanogaster models of Parkinson's disease. *Genome,* 60(1): 46-54.

Meulener, M., Whitworth, A.J., Armstrong-Gold, C.E., Rizzu, P., Heutink, P., Wes, P.D., Wes, P.D., Pallanck, L.J., & Bonini, N.M., (2005). Drosophila DJ-1 mutants are selectively sensitive to environmental toxins associated with Parkinson's disease. *Current Biology,* 15(17): 1572-1577.

Miura, E., Hasegawa, T., Konno, M., Suzuki, M., Sugeno, N., Fujikake, N., Geisler, S., Tabuchi, M., Oshima, R., Kikuchi, A., Baba, T., Wada, K., Nagai, Y., Takeda, A., & Aoki, M. (2014). VPS35 dysfunction impairs lysosomal degradation of α-synuclein and exacerbates neurotoxicity in a Drosophila model of Parkinson's disease. *Neurobiology of Disease,* 71: 1-13.

Mizuno, E., Nakamura, M., Agemura, A., Kusumoto, A., Ichiba, M., Kurano, Y., Ichiba, M., Kurano, Y., Muroya, S., & Sano A., (2007). Brain-specific transcript variants of 5′ and 3′ ends of mouse VPS13A and VPS13C. *Biochemical and Biophysical Research Communications,* 353(4): 902-907.

Moon, H.E., & Paek, S.H., (2015). Mitochondrial dysfunction in Parkinson's disease. *Experimental Neurobiology,* 24(2): 103-116.

Muqit, M.M., Abou-Sleiman, P.M., Saurin, A.T., Harvey, K., Gandhi, S., Deas, E., Eaton, S., Payne, M.D., Venner, K., Matilla, A., Healy, D.G., Gilks W.P., Lees A.J., Holton, J., Revesz, T., Parker, P.J., Harvey, R.J., Wood, N.W., & Latchmn D.S., (2006). Altered cleavage and localization of PINK1 to aggresomes in the presence of proteasomal stress. *Journal of Neurochemistry,* 98(1): 156-169.

Murphy, M.P., (2009). How mitochondria produce reactive oxygen species. *Biochemical Journal,* 417(1): 1-13.

Murthy, M.N., & Ramachandra, N.B., (2017). Prioritization of differentially expressed genes in substantia nigra transcriptomes of Parkinson's disease reveals key protein interactions and pathways. *Meta Gene,* 14: 12-18.

Narendra, D., Tanaka, A., Suen, D.F., & Youle, R.J., (2008). Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *The Journal of Cell Biology,* 183(5): 795-803.

Narendra, D., Walker, J.E., & Youle, R., (2012). Mitochondrial quality control mediated by PINK1 and Parkin: Links to parkinsonism. *Cold Spring Harbor Perspectives in Biology,* 4(11): a011338.

Neckameyer, W.S., (1996). Multiple roles for dopamine in Drosophila development. *Developmental Biology,* 176(2): 209-219.

Neriec, N., & Desplan, C., (2016). From the Eye to the Drain: Development of the Drosophila Visual System. *Current Topics in Developmental Biology,* 116: 247-271.

Ohtsuka, K., & Hata, M., (2000). Mammalian HSP40/DNAJ homologues: Cloning of novel cDNAs and a proposal for their classification and nomenclature. *Cell Stress and Chaperones,* 5(2): 98-112.

Otto, G. P., Razi, M., Morvan, J., Stenner, F., & Tooze, S.A., (2010). A novel syntaxin 6-Interacting protein, SHIP164, regulates syntaxin 6-Dependent sorting from early endosomes. *Traffic,* 11(5): 688-705.

Outeiro, T.F., Kontopoulos, E., Altmann, S.M., Kufareva, I., Strathearn, K.E., Amore, A. M., Amore, A.M., Volk, C.B., Maxwell, M.M., Rochet, J.C., McLean, P.J., Young, A.B., Abagyan,R., Feany, M.B., Hyman, B.T., & Kazantsev, A.G. (2007). Sirtuin 2 inhibitors rescue alpha-synuclein-mediated toxicity in models of Parkinson's disease. *Science,* 317(5837): 516-519.

Paisán-Ruíz, C., Jain, S., Evans, E. W., Gilks, W. P., Simón, J., van der Brug, M., De Munain, A.L., Aparicio, S., Gil, A.M., Khan, N., Johnson, J., Martinez, J.R., Nicholl, D., Martí Carrera, I., Pena, A.S., de Silva. R., Lees, A., Martí-Massó, J.F., Pérez-Tur, J., Wood, N.W., & Singleton, A.B., (2004). Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron,* 44(4): 595-600.

Pandey, U.B., & Nichols, C.D., (2011). Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery. *Pharmacological Reviews,* 63(2): 411-436.

Park, J., Lee, S. B., Lee, S., Kim, Y., Song, S., Kim, S., Bae, E., Kim, J., Shong, M., Kim, J.M., & Chung, J., (2006). Mitochondrial dysfunction in drosophila PINK1 mutants is complemented by parkin. *Nature,* 441(7097): 1157-1161.

Perier, C., Bové, J., Dehay, B., Jackson-Lewis, V., Rabinovitch, P.S., Przedborski, S., & Vila, M., (2010). Apoptosis-inducing factor deficiency sensitizes dopaminergic neurons to parkinsonian neurotoxins. *Annals of Neurology, 68*(2), 184-192.

Perrett, R.M., Alexopoulou, Z., & Tofaris, G.K., (2015). The endosomal pathway in Parkinson's disease. *Molecular and Cellular Neuroscience, 66*, 21-28.

Pesah, Y., Pham, T., Burgess, H., Middlebrooks, B., Verstreken, P., Zhou, Y., Harding, M., Bellen, H., & Mardon, G., (2004). Drosophila parkin mutants have decreased mass and cell size and increased sensitivity to oxygen radical stress. *Development,* 131(9), 2183-2194.

Pesah, Y., Burgess, H., Middlebrooks, B., Ronningen, K., Prosser, J., Tirunagaru, V., Zysk, J., & Mardon, G., (2005). Whole-mount analysis reveals normal numbers of dopaminergic neurons following misexpression of alpha-Synuclein in Drosophila. *Genesis,* 41(4): 154-159.

Phelps, C.B., & Brand, A.H., (1998). Ectopic gene expression in DrosophilaUsing GAL4 system. *Methods,* 14(4): 367-379.

Pickrell, A.M., & Youle, R.J., (2015). The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease. *Neuron,* 85(2): 257-273.

Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E.S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W.G., Lazzarini, A.M., Duvoisin, R.C., Di Iorio, G., Golbe, L.I., & Nussbaum, R.L., (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *science*, 276(5321): 2045-2047.

Quadri, M., Fang, M., Picillo, M., Olgiati, S., Breedveld, G. J., Graafland, J., Wu, B., Xu, F., Erro, R., Amboni, M., Pappatà, S., Quarantelli, M., Annesi, G., Quattrone, A., Chien, H.F., Barbosa, E.R.,Oostra, B.A., Barone, P., Wang, J., & Bonifati, V., (2013). Mutation in the SYNJ1 gene associated with autosomal recessive, early-onset Parkinsonism. *Human Mutation,* 34(9): 1208-1215.

Ramirez, A., Heimbach, A., Gründemann, J., Stiller, B., Hampshire, D., Cid, L.P., Goebel, I., Mubaidin, A.F., Wriekat, A.L., Roeper, J., Al-Din, A., Hillmer, A.M., Karsak, M., Liss, B., Woods, C.G., Behrens, M.I., & Kubisch, C. (2006). Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. *Nature Genetics,* 38(10): 1184-1191.

Redding, K., Brickner, J.H., Marschall, L.G., Nichols, J.W., & Fuller, R.S., (1996). Allele-specific suppression of a defective trans-golgi network (TGN) localization signal in Kex2p identifies three genes involved in localization of TGN transmembrane proteins. *Molecular and Cellular Biology,* 16(11): 6208-6217.

Rendón, W.O., Martínez-Alonso, E., Tomás, M., Martínez-Martínez, N., & Martínez-Menárguez, J.A., (2013). Golgi fragmentation is rab and SNARE dependent in cellular models of Parkinson's disease. *Histochemistry and Cell Biology,* 139(5): 671-684.

Ross, J., Olson, L., & Coppotelli, G., (2015). Mitochondrial and Ubiquitin Proteasome System Dysfunction in Ageing and Disease: Two Sides of the Same Coin? *International Journal of Molecular Sciences,* 16(8): 19458-19476.

Rüb, C., Wilkening, A., & Voos, W., (2017). Mitochondrial quality control by the Pink1/Parkin system. *Cell and Tissue Research,* 367(1): 111-123.

Satake, W., Nakabayashi, Y., Mizuta, I., Hirota, Y., Ito, C., Kubo, M., Kawaguchi, T., Tsunoda, T., Watanabe, M., Takeda, A., Tomiyama, H., Nakashima, K., Hasegawa, K., Obata, F., Yoshikawa, T., Kawakami, H., Sakoda, S., Yamamoto, M., Hattori, N., Murata, M., Nakamura, Y., & Toda, T. (2009). Genome-wide association study identifies common variants at four loci as genetic risk factors for parkinson's disease. *Nature Genetics,* 41(12): 1303-1307.

Sauve, V., Lilov, A., Seirafi, M., Vranas, M., Rasool, S., Kozlov, G., Sprules, T., Wang, J., Trempe, J.F., & Gehring, K., (2015). A ubl/ubiquitin switch in the activation of Parkin. *The EMBO Journal,* 34(20): 2492-2505.

Savitt, J.M., Dawson, V.L., & Dawson, T.M., (2006). Diagnosis and treatment of parkinson disease: Molecules to medicine. *The Journal of Clinical Investigation,* 116(7): 1744-1754.

Schapira, A.H., & Jenner, P., (2011). Etiology and pathogenesis of Parkinson's disease. *Movement Disorders,* 26(6): 1049-1055.

Schneider, C.A., Rasband, W.S., & Eliceiri, K.W., (2012). NIH image to ImageJ: 25 years of image analysis. *Nature Methods,* 9(7): 671-675.

Seirafi, M., Kozlov, G., & Gehring, K., (2015). Parkin structure and function. *The FEBS Journal,* 282(11): 2076-2088.

Shah, R., Ohashi, T., Erickson, H.P., & Oas, T.G., (2017). Spontaneous unfolding-refolding of fibronectin type III domains assayed by thiol exchange: thermodynamic stability correlates with rates of unfolding rather than folding. *The Journal of Biological Chemistry,* 292(3): 955-966.

Shimura, H., Hattori, N., Kubo, S., Mizuno, Y., Asakawa, S., Minoshima, S., Shimizu, N., Iwai, K., Chiba, T., Tanaka, K., & Suzuki, T., (2000). Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. *Nature Genetics,* 25(3): 302-305.

Silvestri, L., Caputo, V., Bellacchio, E., Atorino, L., Dallapiccola, B., Valente, E. M., & Casari, G., (2005). Mitochondrial import and enzymatic activity of PINK1 mutants associated to recessive parkinsonism. *Human Molecular Genetics,* 14(22): 3477-3492.

Smith, S., Boitz, J., Chidambaram, E.S., Chatterjee, A., Ait-Tihyaty, M., Ullman, B., & Jardim, A., (2016). The cystathionine-β-synthase domains on the guanosine 5′′-monophosphate reductase and inosine 5′-monophosphate dehydrogenase enzymes from Leishmania regulate enzymatic activity in response to guanylate and adenylate nucleotide levels. *Molecular Microbiology,* 100(5): 824-840.

Starkov, A.A., (2008). The role of mitochondria in reactive oxygen species metabolism and signaling. *Annals of the New York Academy of Sciences,* 1147(1): 37-52.

Strauss, K.M., Martins, L.M., Plun-Favreau, H., Marx, F.P., Kautzmann, S., Berg, D., Gasser, T., Wszolek, Z., Müller, T., Bornemann A., Wolburg H., Downward, J., Riess, O., Schulz, J.B., & Krüger, R. (2005). Loss of function mutations in the gene encoding omi/HtrA2 in Parkinson's disease. *Human Molecular Genetics,* 14(15): 2099-2111.

Strokin, M., Seburn, K.L., Cox, G.A., Martens, K.A., & Reiser, G., (2012). Severe disturbance in the Ca2 signaling in astrocytes from mouse models of human infantile neuroaxonal dystrophy with mutated Pla2g6. *Human Molecular Genetics,* 21(12): 2807-2814.

Subramaniam, S.R., Vergnes, L., Franich, N.R., Reue, K., & Chesselet, M., (2014). Region specific mitochondrial impairment in mice with widespread overexpression of alpha-synuclein. *Neurobiology of Disease,* 70: 204-213.

Tao, X., & Tong, L., (2003). Crystal structure of human DJ-1, a protein associated with early onset Parkinson's disease. *The Journal of Biological Chemistry,* 278(33): 31372-31379.

Thomas, B.J., & Wassarman, D.A., (1999). A fly's eye view of biology. *Trends in Genetics,* 15(5): 184-190.

Thomas, B., & Beal, M.F., (2011). The genes of Parkinson's disease. *Scientist,* 25(2): 1

Todd, A.M., & Staveley, B.E., (2004). Novel assay and analysis for measuring climbing ability in Drosophila. *Drosophila Information Services, 87*: 101-107.

Todd, A.M., & Staveley, B.E., (2008). Pink1 suppresses alpha-synuclein-induced phenotypes in a Drosophila model of Parkinson's disease. *Genome,* 51(12): 1040-1046.

Tomemori, Y., Ichiba, M., Kusumoto, A., Mizuno, E., Sato, D., Muroya, S., Nakamura, M, Kawaguchi, H., Yoshida, H., Ueno, S.I., Nakao, K., Nakamura, K., Aiba, A., Katsuki, M., & Sano, A. (2005). A gene-targeted mouse model for chorea-acanthocytosis. *Journal of Neurochemistry,* 92(4): 759-766.

Tsunemi, T., Hamada, K., & Krainc, D., (2014). ATP13A2/PARK9 regulates secretion of exosomes and α-synuclein. *The Journal of Neuroscience,* 34(46): 15281-15287.

Valente, E.M., Abou-Sleiman, P.M., Caputo, V., Muqit, M.M., Harvey, K., Gispert, S., Ali, Z., Del Turco, D., Bentivoglio, A.R., Healy, D.G., Albanese, A., Nussbaum, R., González-Maldonado, R., Deller, T., Salvi, S., Cortelli, P., Gilks, W.P., Latchman, D.S., Harvey, R.J., Dallapiccola, B., Auburger, G., & Wood, N.W. (2004). Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science,* 304(5674): 1158-1160.

van der Merwe, C., Jalali Sefid Dashti, Z., Christoffels, A., Loos, B., & Bardien, S., (2015). Evidence for a common biological pathway linking three Parkinson's disease-causing genes: Parkin, PINK1 and DJ-1. *European Journal of Neuroscience,* 41(9): 1113-1125.

Van Swinderen, B., & Andretic, R., (2011). Dopamine in Drosophila: Setting arousal thresholds in a miniature brain. *Proceedings.Biological Sciences,* 278(1707): 906-913.

Velayos-Baeza, A., Vettori, A., Copley, R.R., Dobson-Stone, C., & Monaco, A., (2004). Analysis of the human VPS13 gene family. *Genomics,* 84(3): 536-549.

Venderova, K., Kabbach, G., Abdel-Messih, E., Zhang, Y., Parks, R. J., Imai, Y., Gehrke, S., Ngsee, J., LaVoie, M.J., Slack, R.S., Rao, Y., Zhang, Z,, Lu, B., Haque, M.E., & Park, D.S., (2009). 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,* 18(22): 4390-4404.

Videnovic, A., & Golombek, D., (2017). Circadian Dysregulation in Parkinson's Disease. *Neurobiology of Sleep and Circadian Rhythms,* 2: 53-58.

Vilariño-Güell, C., Rajput, A., Milnerwood, A.J., Shah, B., Szu-Tu, C., Trinh, J., Yu, I., Encarnacion, M., Munsie, L.N., Tapia, L., Gustavsson, E.K., Chou, P., Tatarnikov, I., Evans, D.M., Pishotta, F.T., Volta, M., Beccano-Kelly, D., Thompson, C., Lin, M.K., Sherman, H.E., Han, H.J., Guenther, B.L., Wasserman, W.W., Bernard, V., Ross, C.J., Appel-Cresswell, S., Stoessl, A.J., Robinson, C.A., Dickson, D.W., Ross, O.A., Wszolek, Z.K., Aasly, J.O., Wu, R.M., Hentati, F., Gibson, R.A., McPherson, P.S., Girard, M., Rajput, M., Rajput, A.H., & Farrer, M.J., (2013). DNAJC13 mutations in parkinson disease. *Human Molecular Genetics,* 23(7): 1794-1801.

Wang, C., Lu, R., Ouyang, X., Ho, M.W., Chia, W., Yu, F., & Lim K.L., (2007). Drosophila overexpressing parkin R275W mutant exhibits dopaminergic neuron degeneration and mitochondrial abnormalities. *Journal of Neuroscience,* 27(32): 8563-8570.

Wang, L., Karpac, J., & Jasper, H., (2014). Promoting longevity by maintaining metabolic and proliferative homeostasis. *Journal of Experimental Biology,* 217(1): 109-118.

Wang, S., & Bellen, H.J., (2015). The retromer complex in development and disease. *Development,* 142(14): 2392-2396.

Wang, W., Wang, X., Fujioka, H., Hoppel, C., Whone, A.L., Caldwell, M.A., Cullen, P.J., Liu, J., & Zhu, X., (2016). Parkinson's disease-associated mutant VPS35 causes mitochondrial dysfunction by recycling DLP1 complexes. *Nature Medicine,* 22(1): 54-63.

Whitworth, A.J., Theodore, D.A., Greene, J.C., Benes, H., Wes, P.D., & Pallanck, L.J., (2005). Increased glutathione S-transferase activity rescues dopaminergic neuron loss in a Drosophila model of Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America,* 102(22): 8024-8029.

Whitworth, A.J., Wes, P.D., & Pallanck, L.J., (2006). Drosophila models pioneer a new approach to drug discovery for Parkinson's disease. *Drug Discovery Today,* 11(3-4): 119-126.

Winklhofer, K.F., & Haass, C., (2010). Mitochondrial dysfunction in Parkinson's disease. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease,* 1802(1): 29-44.

Wood-Kaczmar, A., Gandhi, S., Yao, Z., Abramov, A. S., Miljan, E. A., Keen, G., Stanyer, L., Hargreaves, I., Klupsch, K., Deas, E., Downward, J., Mansfield, L., Jat, P., Taylor, J., Heales, S., Duchen, M.R., Latchman, D., Tabrizi, S.J., & Wood, N.W. (2008). PINK1 is necessary for long term survival and mitochondrial function in human dopaminergic neurons. *PloS One,* 3(6): e2455.

Xilouri, M., & Stefanis, L., (2011). Autophagic pathways in Parkinson disease and related disorders. *Expert reviews in Molecular Medicine,* 13: e8

Xiong, Y., Dawson, T.M., & Dawson, V.L., (2017). Models of LRRK2-associated Parkinson's disease. *Advances in Neurobiology* 14:163-191.

Yang, Y., Gehrke, S., Haque, M.E., Imai, Y., Kosek, J., Yang, L., Beal, M.F., Nishimura I, Wakamatsu, K., Ito, S., & Takahashi R., & Lu, B., (2005). Inactivation of Drosophila DJ-1 leads to impairments of oxidative stress response and phosphatidylinositol 3-kinase/akt signaling. *Proceedings of the National Academy of Sciences of the United States of America,* 102(38): 13670-13675.

Yeh, E., Gustafson, K., & Boulianne, G.L., (1995). Green fluorescent protein as a vital marker and reporter of gene expression in Drosophila. *Proceedings of the National Academy of Sciences of the United States of America,* 92(15): 7036-7040.

Zavodszky, E., Seaman, M.N., Moreau, K., Jimenez-Sanchez, M., Breusegem, S.Y., Harbour, M.E., & Rubinsztein D.C., (2014). Mutation in VPS35 associated with Parkinson's disease impairs WASH complex association and inhibits autophagy. *Nature Communications,* 5: 3828.

Zhang, R., Evans, G., Rotella, F.J., Westbrook, E.M., Beno, D., Huberman, E., Joachimiak, A., & Collart F.R., (1999). Characteristics and crystal structure of bacterial inosine-5 '-monophosphate dehydrogenase. *Biochemistry,* 38(15): 4691-4700.

Zhao, T., Severijnen, L., van der Weiden, M., Zheng, P.P., Oostra, B.A., Hukema, R.K., Willemsen R., Kros J.M., & Bonifati, V., (2013). FBXO7 immunoreactivity in α-

synuclein-containing inclusions in Parkinson disease and multiple system atrophy. *Journal of Neuropathology and Experimental Neurology,* 72(6): 482-488.

Zimprich, A., Benet-Pagès, A., Struhal, W., Graf, E., Eck, S.H., Offman, M.N., Haubenberger, D., Spielberger, S., Schulte, E.C., Lichtner, P., Rossle, S.C., Klopp, N., Wolf, E., Seppi, K., Pirker, W., Presslauer, S., Mollenhauer, B., Katzenschlager, R., Foki, T., Hotzy, C., Reinthaler, E., Harutyunyan A., Kralovics, R., Peters, A., Zimprich, F., Brücke, T., Poewe, W., Auff, E., Trenkwalder, C., Rost, B., Ransmayr, G., Winkelmann, J., Meitinger, T., & Strom, T.M., (2011). A mutation in VPS35, encoding a subunit of the retromer complex, causes late-onset Parkinson disease. *American Journal of Human Genetics,* 89(1): 168-175.