

## **S.I. : Genetic pathways to Neurodegeneration**

### **Pathways to Neurodegeneration: Lessons Learnt From Unbiased Genetic Screens in *Drosophila***

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#### **Neurodegeneration**

Neurodegenerative diseases (NDD) are enervating disorders that occur as a result of progressive loss of selective type of neurons, leading to a diverse set of symptoms. These symptoms manifest as physical, behavioral or cognitive limitations. Examples of neurodegenerative diseases include Parkinson's disease, Alzheimer's disease, and Amyotrophic lateral sclerosis. Parkinson's disease is caused by the loss of dopaminergic neurons in substantia nigra with clinical manifestations including resting tremor, muscular rigidity, bradykinesia, and postural instability. In contrast, Alzheimer's disease is caused by the loss of central cholinergic neurons with clinical symptoms including progressive dementia. The neurodegenerative disorders can be familial or sporadic, that is, stemming from either genetic or environmental or aging factors. Apart from genetic inheritability in some cases, unhealthy lifestyle and increasing aging population are contributing to the global neurodegenerative diseases burden at an alarming rate (Brown et al. 2005; Salvadores et al. 2017). Hence, due to the multifactorial nature of these diseases, it is imperative to understand the causes and the mechanisms behind pathology of specific diseases, so that effective treatments can be designed.

While pathological features are specific for a particular neurodegenerative disease, there are several hallmarks that associate with most of the neurodegenerative diseases. One such key hallmark is an abnormal accumulation of misfolded protein aggregates (proteopathies) (Sweeney et al. 2017) (Weydt and La Spada 2006). For example, presence of Amyloid beta (A $\beta$ ) protein aggregates in case of Alzheimer's disease (Bahmanyar et al. 1987; Selkoe 1994),  $\alpha$ -synuclein in case of Parkinson's disease (Breydo et al. 2012; Uversky 2007) and mutant Huntingtin protein in case of Huntington's disease (Landles et al. 2010; Busch et al. 2003). These aggregates become

neurotoxic and perturb vital cellular processes, ultimately causing loss of synapses and neurons (Demuro et al. 2005; Ugalde et al. 2016; Roostae et al. 2013). Other important hallmarks include impaired protein degradation machinery (Kim et al. 2017; Tai et al. 2012) (Tydlacka et al. 2008; Ciechanover and Kwon 2015), aberrant gene expression (Li et al. 2014), oxidative stress, and mitochondrial dysfunction (Lin and Beal 2006; Guo et al. 2013). Neuroinflammation is another pathogenic hallmark of most neurodegenerative diseases, and involves activation of microglial cells, followed by release of the pro-inflammatory mediators such as cytokines, chemokines, interleukins and ROS (Frank-Cannon et al. 2009; Ransohoff 2016) .

Notably, as observed with several neurodegenerative diseases such as ALS and Parkinson's disease, both the familial and sporadic forms of the disease show similar hallmarks and disease progression (Papapetropoulos et al. 2007; Carr et al. 2003; Talbot 2011). The familial aspect of neurodegenerative diseases gives an opportunity to determine the disease associated genes, and further, pinpoint the roles of individual mutations and pathways involved. Importantly, the use of a model organism for neurodegenerative diseases allows us to probe into the biological function of genes and pathogenic mechanism *in vivo*. Indeed, the relevant animal models can help us to track and understand disease progression bit-by-bit, and even aid in conducting high-throughput compound screening towards drug development. Presently, flies (*Drosophila melanogaster*) (Bilen and Bonini 2005; Venken et al. 2011), worms (*C. elegans*) (Wang et al. 2017; Li and Le 2013), and mice (*Mus musculus*) (Havekes and Abel 2009; Trancikova et al. 2011) are the major model organisms that have contributed to study of neuronal diseases.

### ***Drosophila* as a model system for neurodegenerative diseases**

*Drosophila* has been an important model organism to study neurodegenerative diseases. Some of the major advantages of this model include short generation time, small genome size, low genetic redundancy and availability of tools for genetic manipulation. Fly genome comprises about 16000 genes and about 8000 fly genes are conserved in human (Shih et al. 2015; Wangler et al. 2015). More importantly, unparalleled tools for genetic manipulation of these genes in flies allow the study of human diseases using forward and reverse genetics approaches (Lenz et al. 2013). The forward genetic screen involves introducing random genome-wide mutations, leading to the generation of flies with aberrant phenotypes. These mutations are then mapped to the genome leading to the discovery of the genes involved in the process under study. In contrast, the reverse genetics involves targeted mutagenesis of the known gene and is aimed at deciphering its biological function *in vivo*. Most importantly, similarities in the nervous system function and organization allow the use of *Drosophila* in exploring the mechanisms of neuronal function, survival and degeneration *in vivo* (Bellen et al. 2010). Indeed, the flies bearing mutations in genes whose human homologs are linked to NDD often develop neurodegenerative phenotypes that are strikingly similar to humans. Reviewed in (Sharma et al. 2017; Kim et al. 2017; Hewitt and Whitworth 2017; Lewis and Smith 2016).

Various neurodegenerative diseases have been modeled and studied successfully in flies such as Parkinson's disease, Alzheimer's disease, Huntington's disease and Amyotrophic Lateral Sclerosis. These studies have been reviewed extensively in the past (Lu and Vogel 2009; Bilen and Bonini 2005; Zoghbi and Botas 2002; Pandey and Nichols 2011). For instance, the models for Parkinson's disease include  $\alpha$ -Syn models (mutant flies show presence of Lewy body-like aggregates, degeneration of dopaminergic neurons and defects in locomotion), parkin models (mutant flies show mitochondrial aberrations, apoptotic muscle degeneration, and reduced life span), DJ-1 models (mutant flies show hypersensitivity to oxidative stress) and Pink1 models (mutant flies show male sterility, apoptotic muscle degeneration, aberrant mitochondrial morphology and increased oxidative stress). In case of Alzheimer's disease, most of the models show A $\beta$  peptide-induced amyloid formation, leading to neurodegeneration. The gene mutations involve either loss of AD-associated fly homologs like APPL and Psn or expression of mutant human homologs like BACE. Even tau-induced neurodegeneration has been generated by expression of mutant human tau or loss of *Drosophila* tau. Further, many polyQ disease models such as SCA-3, SCA-1, HD, and SBMA demonstrating retinal degeneration have also been developed in flies. In fact, the length of the polyQ tract has been found to correlate with severity of neurodegeneration. Thus, various features of neurodegenerative diseases that have been successfully modeled in *Drosophila* include accumulation of abnormal aggregates of the mutant proteins, proteotoxicity, and mitochondrial dysfunction, further leading to neuronal dysfunction and demise. In this review, we will focus on the recent discoveries about genes and pathways that lead to neurodegeneration, made using forward genetic screens in flies.

### **Unbiased forward genetic screens in flies to study neuro-maintenance**

The significance of genetic screens in *Drosophila* is well demonstrated by the key insights acquired into a number of biological processes such as development, neuronal function, behavior and metabolism (Gaytán de Ayala Alonso et al. 2007; Axelrod et al. 2015; Reis et al. 2010). Similarly, a growing body of evidence validates the enormous potential of genetic tools that facilitate forward and reverse genetic screens in deciphering pathways and complex mechanisms that are involved in neuro-protection and neurodegeneration (Lenz et al. 2013; Jaiswal et al. 2012; Bilen and Bonini 2005; Bellen and Yamamoto 2015; Lessing and Bonini 2009). Several forward and reverse genetic screens have facilitated the identification of novel genes linked to neurodegeneration and provided insight into the pathology of complex neurodegenerative disorders such Alzheimer's disease, Parkinson's disease and Amyotrophic Lateral Sclerosis (Lu and Vogel 2009; Debattisti and Scorrano 2013; Hirth 2010). For instance, an ethyl methanesulphonate (EMS) forward genetic screen carried out to identify genes involved in the neuronal health and synapse development (Mehta et al. 2005) in flies eventually led to the identification of novel gene mutations linked to Autosomal Recessive Spastic Ataxia with Leukoencephalopathy (ARSAL) (OMIM #611390) (Bayat et al. 2012). The deleterious mutations in the *Drosophila Mitochondrial Methionine-tRNA Synthetase*, *Aats-met* gene cause retinal degeneration, reduced lifespan, muscle degeneration, impaired cell proliferation, increased ROS

and reduced mitochondrial respiration in the mutant flies. Further, the expression of human homolog, *MARS2*, could rescue the phenotype of *aats-met* mutant flies, suggesting that *aats-met* and *MARS2* are orthologs. Interestingly, authors found *MARS2* to be present in a 3.3 Mb long candidate gene interval previously linked to neurometabolic disease ARSAL (Thiffault et al. 2006). The sequence analysis of several selected ARSAL patients uncovered the occurrence of *MARS2* mutations involving duplication events in these patients, linking the association of *MARS2* gene with the disease. The diagnosis was first made in 54 affected French-Canadian patients belonging to 38 families, with all patients carrying complex *MARS2* genomic rearrangements. Further, in corroboration with the defects in flies, the ARSAL patient cells also exhibit increased ROS levels and reduced mitochondrial activity and cell proliferation rate. Hence, such integrative approaches demonstrate a bidirectional synergism between humans and flies and can fast-track the gene discovery and disease diagnosis, if supported by collaborative scientific efforts between researchers and clinicians.

Another forward genetic screen, which was designed to identify genes required for the development, function, and maintenance of the nervous system in flies, identified mutations in 165 fly genes on the *Drosophila* X-chromosome (Yamamoto et al. 2014). Remarkably, 93% of these fly genes have human homologs, of which 31% are linked to human diseases, including a diverse set of neurodegenerative diseases. These included *Marf* (*MFN2* in humans), *sicily* (*C8ORF38* in humans), and *cacophony* (*CACNA1A* in humans). Mutations in *Marf* are known to cause *Charcot-Marie-Tooth disease* (CMT) Type 2A2A and CMT Type 2A2B (Calvo et al. 2009; Chung et al. 2006; Polke et al. 2011), while mutations in *sicily* are linked to Leigh syndrome (Pagliarini et al. 2008; Bianciardi et al. 2016), and *cacophony* are linked to Spinocerebellar ataxia 6 (Ishikawa et al. 1997) and episodic ataxia, type 2 (Labrum et al. 2009). *swisscheese* is another fly gene that had previously been associated with progressive degeneration of adult nervous system in flies (Kretzschmar et al. 1997). The human homolog of *swisscheese*, *PNPLA6*, has now been linked to various neurodegenerative diseases such as Spastic paraplegia 39, autosomal recessive (Rainier et al. 2008), Boucher-Neuhauser syndrome (Synofzik et al. 2014), and Laurence-Moon syndrome (Hufnagel et al. 2015). In fact, the role of *swisscheese* in flies in neuronal ensheathment and function has only recently been discovered (Dutta et al. 2016). The *Drosophila* genetic screens have also identified genes such as *wasted away*, a *Drosophila* mutation in triosephosphate isomerase, involved in paralysis, neurodegeneration, and early death. (Gnerer et al. 2006) Another example includes the discovery of *ATPIA3* being linked with Dystonia 12, known as rapid-onset dystonia-parkinsonism (Kaneko et al. 2014). This demonstrates that unbiased genetic screens are a reliable genetic tool for isolating mutations in genes necessary for neuronal survival. In fact, the genetic screen by Yamamoto et al. paved the way for identification of several novel disease-linked-genes and disease diagnoses (discussed later in the review) done over a short period of 2-3 years from the screen.

### ***Discovery of new diseases linked genes***

In recent past, the development of whole-genome sequencing has tremendously facilitated the identification of novel mutations that are responsible for disease phenotypes and subsequent studies in model organisms helps in understanding the pathogenic mechanism and gene function. However, sequencing of any patient's genome gives rise to the identification of a large number of variants that appear to be deleterious as per bioinformatics analysis (Foong et al. 2015). This provides a real challenge in the identification of a variant that is responsible for the disease under investigation (Chakravarti et al. 2013). In such cases, further diagnosis can be supported by phenotypic information available from genetic studies carried out in model organisms (Wangler et al. 2017; Yoon et al. 2017). As observed, human orthologs of several fly genes, whose loss is known to cause neurodegeneration, are linked to human NDD. This strongly suggests that genetic information from flies can be helpful in the prioritization of variants identified in patient genome for further validation. As a first step in this approach, the unbiased forward genetic screens generate mutant flies with desired neuronal phenotypes, the genetic loci responsible for the phenotype are mapped, and human homologs are determined. The potential disease-associated human variants are mined by drawing the genotypic and phenotypic comparisons between the human patients and the mutant flies. Finally, the rescue experiments are performed by expressing cDNA of human homolog of the fly gene in the corresponding fly mutant to test the conservation of gene function between fly and human, and to confirm its disease causation. This approach of the simultaneous exploration of genomic and functional/phenotypic parallels between the flies and humans studies has identified novel gene-disease links. For example, deleterious mutations in the genes *DNM2* and *LRSAMI* are linked to Charcot-Marie-Tooth Neuropathy Type 2 (OMIM #614436), the *CRX* gene mutations, previous linked to childhood vision loss, Leber congenital amaurosis, and Cone-rod retinal dystrophy-2 are now also linked to Bull's eye Maculopathy (OMIM #153870)(Yamamoto et al. 2014). Further, the screen by Yamamoto et al. isolated a mutant line showing a small brain phenotype. The mutations were mapped to a *Dankle2* gene, A homolog of human gene *ANKLE2*. An inquiry of WES data from the patients displaying comparable neurological defects led to the revelation of mutations in *ANKLE2* (human homolog of *dankle2*) in patients with microcephaly. Microcephaly is a developmental disorder where defects in brain development results in a smaller head size in humans. Potential deleterious alleles in *ANKLE2*, responsible for autosomal recessive primary microcephaly 16 (MIM# 616681) were identified in two siblings of a family. The disease association with *ANKLE2* was further backed by the successful rescue of lethality, brain size, and apoptosis in *dAnkle2* mutants by the human *ANKLE2* (Yamamoto et al. 2014). With similar strategy Nardilysin, another gene identified through the genetic screen (Yamamoto et al. 2014), was linked to human neurodegeneration. The *Drosophila nardilysin* (*dNrd1*) mutant flies showed progressive degeneration of photoreceptors and loss of synaptic transmission. Yoon et al. discovered that Nardilysin is a co-chaperone and ensures proper refolding of  $\alpha$ -ketoglutarate dehydrogenase (OGDH), a rate-limiting enzyme in the TCA cycle (Yoon et al. 2017). A neurodegenerative phenotype in *dNrd1* or *dOgdh* in flies and severe neuronal defects in *mNrd1* knockout mice (Ohno et al. 2009), led the authors to search for possible neurodegeneration associated variants in the orthologous human genes. Indeed, a

reference on <https://genematcher.org> for WES sequencing of a patient with severe global developmental delay and ataxia led to the identification of a homozygous truncating variant in *NRD1*. This was achieved by systematic elimination of gene mutations reported to be benign, and zeroing onto *NRD1* or *OGDHL* as candidate genes. Furthermore, the patients bearing homozygous mutations in *OGDH* were also discovered and displayed similar phenotypes to *NRD1* variants, such as early developmental defects, progressive neurodegeneration, microcephaly, and ataxia indicating their disease association.

Apart from the unbiased forward genetic screens, genome-wide RNAi screens have also successfully been employed in flies to identify the components of specific cellular processes (Zhang et al. 2006). In a notable study, Neely et al. performed genome-wide neuronal-specific RNAi knock-down in *Drosophila*, and identified a large number of novel genes involved in heat nociception; the sensory nervous system's response to potentially harmful heat stimulus (Neely et al. 2010). The flies were selected on the basis of a well designed behavioral screen that segregated flies on the basis of their response to a noxious (46°C), sub-noxious ( $\leq 39^\circ\text{C}$ ), and non-noxious (31°C) surface. The screen identified several genes such as *straightjacket (stg)*. Flies with *stg* knockdown fail to avoid the noxious temperature. Remarkably, the human homolog of *stg*,  $\alpha 2\delta 3$  (*CACNA2D3*) shows multiple polymorphic variants and a set of pain-sensitivity experiments in healthy volunteers and patients with chronic pain, confirmed the association of these variants in pain perception. Further, the  $\alpha 2\delta$  proteins are present at the extracellular face of presynaptic release sites in the nervous system and the  $\alpha 2\delta$  gene family has been linked to chronic pain (D'Arco et al. 2015), epilepsy (Barclay et al. 2001), and autism (De Rubeis et al. 2014)

### **Flies as a tool for diagnosis using reverse genetics**

In past few years, the human Whole Exome Sequencing (WES) has led the identification of novel gene variants linked with the disease symptoms, while the mechanisms being delineated using reverse genetics in flies. Recurrent de novo *ATAD3A* c.1582C>T variant and biallelic deletion mutations were mined through WES of patients suffering from various neuronal conditions such as hypotonia, global developmental delay and axonal neuropathy (Harel et al. 2016). *ATAD3A* is a mitochondrial membrane protein required for stabilization of nucleoids (He et al. 2007) and in mitochondrial dynamics (Gilquin et al. 2010). Muscle-specific overexpression of the orthologous *Drosophila bor* gene carrying *bor*<sup>R534W</sup> mutation (homologous to human c.1582C>T) lead to drastic reduction in the number of mitochondria, aberrant cristae and an increased autophagy. Further, loss of *bor* resulted in a similar phenotype, suggesting a dominant negative nature of the *bor*<sup>R534W</sup> mutation. Similarly, the fibroblasts of the patients display increased mitophagy, further backing the link between the *ATAD3A* mutations, autophagy and the neuronal defects. Undoubtedly, flies have been instrumental in both identification and *in vivo* validation of novel variants associated with various neurodegenerative. To name a few, deleterious mutations in *KATNB1* (encoding the regulatory subunit of the microtubule-severing enzyme Katanin) being responsible for complex cerebral malformations (Mishra-Gorur et al. 2014), de novo variants in *EBF3* (Early B cell factor

3), a member of Collier/Olf/EBF (COE) family of transcription factors causing neurodevelopmental disorders such as ataxia, CNS malformation and congenital hypotonia (Harms et al. 2017), and role of E3 ubiquitin ligase (ubr3) in Usher's syndrome and MYH9 disorders (Li et al. 2016).

### **Pathways to neurodegeneration underlying mitochondrial dysfunction**

Neurons are highly metabolically active cells that need tremendous amount of energy in the form of ATP for their function and survival (Ames 2000). Hence, neurons are extremely sensitive to energetic balance, or lack of it in and around themselves. Mitochondria are the cellular metabolic hub that are the major source of ATP through oxidative phosphorylation. Other cardinal functions of mitochondria include regulation of cellular Ca<sup>2+</sup> homeostasis, redox balance, iron homeostasis, synthesis of steroids and apoptosis (Bak and Weerapana 2015; Koopman et al. 2010; Glancy and Balaban 2012; Smaili et al. 2000). Inevitably, mitochondrial dysfunction manifests as one of the major hallmark in neurodegenerative diseases (Lin and Beal 2006; Hroudová et al. 2014; Golpich et al. 2017; Keating 2008; Gao et al. 2017; Guo et al. 2013). The role of mitochondria in various NDD has been reviewed extensively (Haun et al. 2013; Chen and Chan 2009; Dawson and Dawson 2017; Martinez-Vicente 2017; Balog et al. 2016). The multitudinous factors for mitochondrial dysfunction include mutations in mitochondrial DNA, or nuclear DNA coding for mitochondrial proteins, or mitochondrial dynamics involving their fission and fusion, impaired mitophagy, perturbed protein import and mitochondrial transport. Increasing number of evidences are revealing the role of mutant mitochondrial proteins in neurodegenerative phenotypes. For example, Mfn2 plays a crucial role in mitochondrial fusion, whereas mutations in the *Mfn2* gene cause the neurodegenerative disease Charcot-Marie-Tooth type 2A (Kijima et al. 2005). Similarly, Pink1 is a mitochondrial serine/threonine-protein kinase involved in mitophagy, but mutations in the *Pink1* gene have been implicated in Parkinson's disease (Kumazawa et al. 2008; Hedrich et al. 2006). Over the years, flies have phenomenally enriched our knowledge about the importance of healthy mitochondria for normal neuronal function. Here we will focus on recent fly studies that reveal novel mechanisms linking mitochondrial dysfunction and neurodegeneration.

### ***Mitochondrial dysfunction and altered iron homeostasis***

A genetic screen aiming to decipher the molecular mechanisms of Pink1, discovered a link between iron accumulation and mitochondrial dysfunction with enhanced ROS and impaired Complex 1 in PD patients. Aconitase (*acon*), was identified as dominant suppressor of Parkinson-related gene Pink1 (Esposito et al. 2013). Inactivation of the Fe-S cluster in *aconitase*, because of increased ROS, leads to disrupted iron homeostasis resulting in a buildup of iron and peroxide that combine to produce hydroxyl radicals and cause mitochondrial dysfunction. Further link of altered Iron homeostasis and neurodegeneration came from studies of a fly homolog of *Frataxin (FXN)*, *fh*. *FXN* is a nuclear-encoded mitochondrial chaperone, and mutations in the *FXN* gene have been associated with Friedreich ataxia (Schöls et al. 2000), a neurodegenerative disorder characterized by progressive loss of nerve cells in the spinal cord, cerebellum and dorsal root ganglia resulting

in gait and limb ataxia. Previous studies have suggested the role of *FXN* mutations in ROS (Wang et al. 2014; Al-Mahdawi et al. 2006; Calabrese et al. 2005) or iron-dependent toxicity (Wang et al. 2014). However, the mechanistic link between *FXN* mutations and iron-mediated neurotoxicity was only recently discovered through the analysis of the mutation in its fly homolog *fh* (Chen, Lin, et al. 2016). *fh* mutant was identified via unbiased forward genetic screen performed to isolate mutations causing neurodegenerative phenotypes described in earlier sections (Yamamoto et al. 2014). The genetic mosaic flies bearing the loss of *fh* in *eyes* display age-dependent photoreceptor degeneration. This phenotype can be rescued by expression of the human *FXN* cDNA, suggesting functional conservation. Further, the *fh* mutants exhibit aberrant mitochondria, reduced electron chain activity, reduced ATP levels, and increased accumulation of iron. Interestingly, no elevation in the oxidative stress was observed. This ruled out any role of oxidative stress in neurodegeneration. At the same time, reducing dietary iron could actively suppress the degeneration in *fh* mutant photoreceptors. This iron toxicity was shown to be linked to enhanced sphingolipid synthesis, which then activates Pdk1/Mef2 signaling (Lee et al. 2012). Interestingly, neurodegeneration in *fh* mutants is suppressed by down-regulation of sphingolipid synthesis or knockdown of *Pdk1* or *Mef2*, while exacerbated by overexpression of *Mef2* (Chen, Lin, et al. 2016). Similar results in mice confirmed the conservation of the mechanistic link between mitochondrial dysfunction, iron accumulation, activation of *Pdk1* /*Mef2* pathway and neurodegeneration (Chen, Ho, et al. 2016). Moreover, sphingolipid levels and PDK1 activity are increased in FRDA patients. Together, these studies clearly demonstrate that overactivation of Pdk1/Mef2 pathway induces degeneration due to loss of *fh* and *fxn*.

### ***Mitochondrial dysfunction and altered oxidative stress***

The forward genetic screen conducted in (Yamamoto et al. 2014) led to the identification of three different fly mutants, *sicily* (NDUFAF6 in humans), *Aats-met* (MARS2 in humans) and *Marf* (*Mitofusin 1* and *2* in humans), all of which exhibit neurodegeneration phenotype. The human homologs of these genes have been linked with the Leigh syndrome (Pagliarini et al. 2008), ARSAL (Bayat et al., 2012) and CMT Type 2A (Kijima et al. 2005) respectively. These mutant flies also show accumulation of lipid droplets, which is correlated to enhanced levels of ROS (Liu et al. 2015; Zhang et al. 2013; Bayat et al. 2012; Sandoval et al. 2014). Extended exposures to ROS have been known to trigger a c-Jun-N-terminal Kinase (JNK) signaling mediated stress response in both *Drosophila* and mammals (Wang et al. 2003). Indeed the flies mutant for *sicily*, *Aats-met* and *Marf* show increased levels of JNK. It was also found that the JNK signaling activated SREBP (Sterol regulatory element-binding protein) in these mutants, leading to increased lipogenesis in neurons. The lipids are then translocated to glia, where they form lipid droplets (LD) (Zhang et al. 2013). The excess of lipid accumulation in the presence of high ROS, leads to their peroxidation, furthering neurodegeneration. Interestingly, suppression of ROS or lipid droplet formation delays degeneration in above mutants. Furthermore, *Ndufs4* (mutant mice, which exhibits increased ROS and neurodegeneration, also accumulate LD in the glia. Similar to flies, presymptomatic antioxidant treatment can efficiently alleviate neurodegeneration in the

*Ndufs4* mutant mouse. This suggests that LD accumulation due to oxidative stress is an evolutionarily conserved phenomenon which promotes neurodegeneration.

### ***An activity-dependent mechanism of neurodegeneration underlying mitochondrial defects***

The above mentioned genetic screen also led to the identification of another mechanism of photoreceptor (PR) degeneration in the mutants that display a decrease in mitochondrial activity without a concomitant presence of oxidative stress. The light-induced photoreceptor degeneration in these mutants occurs due to perturbed  $\text{Ca}^{2+}$  homeostasis leading to excessive rhodopsin accumulation and impaired Rhodopsin recycling. The authors found that mutations in a nuclear-encoded mitochondrial gene, *ppr*, a homolog of human *LRPPRC*, lead to impaired phototransduction cascade causing excessive Rhodopsin1 endocytosis (Jaiswal et al. 2015). The loss of *ppr* causes a decline in mitochondrial RNAs, and reduced ATP levels, without enhanced ROS levels. In contrast, the *sicily* mutants, which show mitochondrial Complex I deficiency and reduced ATP levels along with severely increased ROS levels, exhibit a light-independent PR degeneration that is accelerated by light exposure due to perturbed  $\text{Ca}^{2+}$  homeostasis and impaired Rhodopsin recycling. Similarly, *fh* mutants also displayed severe degeneration in the presence of light due to perturbed  $\text{Ca}^{2+}$  homeostasis and impaired Rhodopsin recycling. Hence, mutations in mitochondrial proteins can be involved in activation of more than one pathological mechanisms, ultimately leading to neurodegeneration.

### ***Activation of TOR pathway and neurodegeneration***

Besides pathways mentioned above for neurodegeneration, a new pathway underlying mitochondrial dysfunction was identified by the study of *dnr1* mutants (Yoon et al. 2017). *dnr1* mutant photoreceptors degenerate through a mechanism independent of light, ROS as well as iron-mediated toxicity. Nardilysin was found to be a co-chaperone that ensures proper refolding of  $\alpha$ -ketoglutarate dehydrogenase (OGDH), a rate-limiting enzyme in the TCA cycle. Loss of *Nrd1* or *Ogdh* leads to an increase in cellular  $\alpha$ -ketoglutarate, a substrate for OGDH. The neurodegenerative phenotype in both *dNrd1* or *dOgdh* mutants is similar, linking increased  $\alpha$ -ketoglutarate with neurodegeneration.  $\alpha$ -ketoglutarate is known to induce mTORC1, which suppresses autophagy. Indeed, loss of *dNrd1* or *dOgdh* showed increased levels of p62, an autophagy substrate suggesting impaired autophagy. Interestingly, *dnr1* mutant flies showed delayed neurodegeneration upon treatment with rapamycin, which induces autophagy. This study not only revealed a novel role for Nardilysin, but also established a novel pathway of neurodegeneration linking aberrations in mitochondrial metabolism, mTORC1 signaling, and impaired autophagy.

### **Concluding remarks:**

Neurodegenerative diseases are one of the most complex sets of human brain disorders. These are debilitating diseases that have long remained difficult to comprehend while explored mainly by means of postpartum examination of the brain. With humongous advancements being in the development of model organisms, such as fruit flies, worms, mice and zebrafish, our understanding

of the development and maintenance of the nervous system has improved significantly in recent past. With about 75% of the human disease genes having fly homologs, *Drosophila* has emerged as an extremely valuable system to identify and validate the biological roles of new disease-associated genes. Apart from the diagnosis of novel neurodegenerative disorders, the simple yet elegant approach of forward and reverse genetic screens have helped in deciphering new players and unique pathways that link mitochondrial function to neurodegeneration. This allows us to understand the factors that can trigger mitochondrial dysfunction, a significant hallmark of NDDs and at the same time design interventions that can restore the health of mitochondria. Apart from being used as a system for genetic dissection of diseases, *Drosophila* can also be used to carry out drug screenings. This ability, combined with the availability of disease models underscores the translational relevance of *Drosophila* models, bringing the bench to bedside targets within reach.

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