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REVIEW ARTICLE

Pathways to neurodegeneration: lessons learnt from unbiased genetic screens in *Drosophila*

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Abstract. Neurodegenerative diseases are a complex set of disorders that are known to be caused by environmental as well as genetic factors. In the recent past, mutations in a large number of genes have been identified that are linked to several neurodegenerative diseases. The pathogenic mechanisms in most of these disorders are unknown. Recently, studies of genes that are linked to neurodegeneration in *Drosophila*, the fruit flies, have contributed significantly to our understanding of mechanisms of neuroprotection and degeneration. In this review, we focus on forward genetic screens in *Drosophila* that helped in identification of novel genes and pathogenic mechanisms linked to neurodegeneration. We also discuss identification of four novel pathways that contribute to neurodegeneration upon mitochondrial dysfunction.

Keywords. neurodegeneration; mitochondrial diseases; *Drosophila*; forward genetic screen.

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, behavioural or cognitive limitations. Examples of NDD include Parkinson's disease (PD), Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS). PD 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, AD is caused by the loss of central cholinergic neurons with clinical symptoms including progressive dementia. The neurodegenerative disorders can be familial or sporadic, i.e., stemming from either genetic or environmental, or ageing factors. Apart from genetic inheritability in some cases, unhealthy lifestyle and increasing ageing population are contributing to the global NDD 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 NDD, there are several hallmarks that associate with most of the NDD. 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 (Aβ) protein aggregates in case of AD (Bahmanyar *et al.* 1987; Selkoe 1994), α-synuclein in case of PD (Uversky 2007; Breydo *et al.* 2012) and mutant Huntingtin protein in case of Huntington's disease (HD) (Busch *et al.* 2003; Landles *et al.* 2010). These aggregates become neurotoxic and perturb vital cellular processes, ultimately causing loss of synapses and neurons (Demuro *et al.* 2005; Roostaee *et al.* 2013; Ugalde *et al.* 2016). Other important hallmarks include impaired protein degradation machinery (Tydlacka *et al.* 2008; Tai *et al.* 2012; Ciechanover and Kwon 2015; Kim *et al.* 2017), 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 NDD, and involves activation of microglial cells, followed by release of the proinflammatory mediators such as cytokines, chemokines, interleukins and reactive oxygen species (ROS) (Frank-Cannon *et al.* 2009; Ransohoff 2016).

Notably, as observed with several NDD such as ALS and PD, both the familial and sporadic forms of the disease show similar hallmarks and disease progression (Carr *et al.* 2003; Papapetropoulos *et al.* 2007; Talbot 2011). The familial aspect of NDD 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 NDD 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 the disease progression gradually, 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 (*Caenorhabditis elegans*) (Li and Le 2013; Wang *et al.* 2017), 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 NDD

Drosophila has been an important model organism to study NDD. 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 16,000 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 genomewide 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, 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 homologues are linked to NDD often develop neurodegenerative phenotypes that are strikingly similar to humans (reviewed in Lewis and Smith 2016; Sharma *et al.* 2017; Kim *et al.* 2017; Hewitt and Whitworth 2017).

Various NDD have been modelled and studied successfully in flies such as PD, AD, HD and ALS. These studies have been reviewed extensively in the past (Zoghbi and Botas 2002; Bilen and Bonini 2005; Lu and Vogel 2009; Pandey and Nichols 2011). For instance, the models for PD include α-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 lifespan), 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 AD, most of the models show Aβ peptide-induced amyloid formation, leading to neurodegeneration. The gene mutations involve either loss of AD-associated fly homologues like beta amyloid protein precursor-like (APPL) and Psn or expression of mutant human homologs like beta-site amyloid precursor protein cleaving enzyme (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 stem cell antigen-3 (SCA-3), SCA-1, HD and spinal-bulbar muscular atrophy (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 NDD that have been successfully modelled in *Drosophila* include accumulation of abnormal aggregates of the mutant proteins, proteotoxicity and mitochondrial dysfunction, leading to neuronal dysfunction and demise. In this review, we 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, behaviour and metabolism (Gaytán de Ayala Alonso *et al.* 2007; Reis *et al.* 2010; Axelrod *et al.* 2015). 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 neuroprotection and neurodegeneration (Bilen and Bonini 2005; Lessing and Bonini 2009; Jaiswal *et al.* 2012; Lenz *et al.* 2013; Bellen and Yamamoto 2015). 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 as AD, PD and ALS (Lu and Vogel 2009; Hirth 2010; Debattisti and Scorrano 2013). 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 *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 orthologues. 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's 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 homologues, of which 31% are linked to human diseases, including a diverse set of NDD. 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 (Chung *et al.* 2006; Calvo *et al.* 2009; 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). Another fly gene *swisscheese* had previously been associated with progressive degeneration of adult nervous system in flies (Kretzschmar *et al.* 1997). The human homolog of *swisscheese* and *PNPLA6* has now been linked to various NDD 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 *ATP1A3* 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.* (2014) paved the way for the 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 helped 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 orthologues 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 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 the human homologues 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 *LRSAM1* 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.* (2014) isolated a mutant line showing small brain phenotype. The mutations were mapped to a *Dankle2* gene, homolog of the human gene *ANKLE2*. An inquiry of whole exome sequencing (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 (OMIM: 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.* (2017) discovered that Nardilysin is a cochaperone and ensures proper refolding of a-ketoglutarate dehydrogenase (OGDH), a rate-limiting enzyme in the tricarboxylic acid cycle (TCA) cycle. 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. Further, the patients bearing homozygous mutations in *OGDH* were also discovered and found to display 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, genomewide 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.* (2010) performed genomewide 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 behavioural screen that segregated flies on the basis of their response to a noxious (46°C), subnoxious (≤39°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*, α2δ3 (*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 α2δ proteins are present at the extracellular face of presynaptic release sites in the nervous system and the α2δ 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 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^{R534W} mutation (homolgous 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^{R534W} 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 Early B cell factor 3 (EBF3), a member of Collier/Olf/EBF (COE) family of transcription factors causing neurodevelopmental disorders such as ataxia, central nervous system 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^{2+} homeostasis, redox balance, iron homeostasis, synthesis of steroids and apoptosis (Smaili *et al.* 2000; Koopman *et al.* 2010; Glancy and Balaban 2012; Bak and Weerapana

2015). Inevitably, mitochondrial dysfunction manifests as one of the major hallmark in NDD (Lin and Beal 2006; Keating 2008; Guo *et al.* 2013; Hroudová *et al.* 2014; Golpich *et al.* 2017; Gao *et al.* 2017). The role of mitochondria in various NDD has been reviewed extensively (Chen and Chan 2009; Haun *et al.* 2013; Balog *et al.* 2016; Dawson and Dawson 2017; Martinez-Vicente 2017). 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 PD (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 between 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 (Calabrese *et al.* 2005; Al-Mahdawi *et al.* 2006; Wang *et al.* 2014) 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 *et al.* 2016b). *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 signalling (Lee *et al.* 2012). Interestingly, neurodegeneration in *fh* mutants is suppressed by downregulation of sphingolipid synthesis or knockdown of *Pdk1* or *Mef2*, while exacerbated by overexpression of *Mef2* (Chen *et al.* 2016b). Similar results in mice confirmed the conservation of the mechanistic link between mitochondrial dysfunction, iron accumulation, activation of *Pdk1*/*Mef2* pathway and neurodegeneration (Chen *et al.* 2016a). 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 by 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 homologues 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 (Bayat *et al.* 2012; Zhang *et al.* 2013; Sandoval *et al.* 2014; Liu *et al.* 2015). Extended exposures to ROS have been known to trigger a c-Jun-N-terminal Kinase (JNK) signallin- 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 signalling 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. Further, *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 mice. 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 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 Ca^{2+} homeostasis and impaired rhodopsin recycling. Similarly, *fh* mutants also displayed severe degeneration in the presence of light due to perturbed 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 *dnrd1* mutants (Yoon *et al.* 2017). *dnrd1* mutant photoreceptors degenerate through a mechanism independent of light, ROS as well as iron-mediated toxicity. Nardilysin was found to be a cochaperone that ensures proper refolding of α-ketoglutarate dehydrogenase (OGDH), a rate-limiting enzyme in the TCA cycle. Loss of *Nrd1* or *OGDH* leads to an increase in cellular a-ketoglutarate, a substrate for OGDH. The neurodegenerative phenotype in both *dNrd1* or *dOgdh* mutants is similar, linking increased α-ketoglutarate with neurodegeneration. α-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, *dnrd1* 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 signalling, and impaired autophagy.

In conclusion, the NDD are one of the most complex set of human brain disorders. These are debilitating diseases being that have long remained difficult to comprehend while explored mainly by means of postpartum examination of the brain. With humongous advancements being made 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 has 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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