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Using *Drosophila* models of Huntington's disease as a translatable tool

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HIGHLIGHTS

- *Drosophila* models of HD display both aggregation and neurodegeneration.
- Human mutant huntingtin (Htt) can be expressed in multiple tissue types in the fly.
- Genetic modifiers of mutant Htt toxicity can be found using *in vivo* genetic screens.
- Candidate genes have been validated for therapeutic potential in HD mouse models.
- Continued screening approaches will likely uncover novel modulators of mutant Htt.

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ABSTRACT

The Huntingtin (Htt) protein is essential for a wealth of intracellular signaling cascades and when mutated, causes multifactorial dysregulation of basic cellular processes. Understanding the contribution to each of these intracellular pathways is essential for the elucidation of mechanisms that drive pathophysiology. Using appropriate models of Huntington's disease (HD) is key to finding the molecular mechanisms that contribute to neurodegeneration. While mouse models and cell lines expressing mutant Htt have been instrumental to HD research, there has been a significant contribution to our understating of the disease from studies utilizing *Drosophila melanogaster*. Flies have an Htt protein, so the endogenous pathways with which it interacts are likely conserved. Transgenic flies engineered to overexpress the human mutant HTT gene display protein aggregation, neurodegeneration, behavioral deficits and a reduced lifespan. The short life span of flies, low cost of maintaining stocks and genetic tools available for *in vivo* manipulation make them ideal for the discovery of new genes that are involved in HD pathology. It is possible to do rapid genome wide screens for enhancers or suppressors of the mutant Htt-mediated phenotype, expressed in specific tissues or neuronal subtypes. However, there likely remain many yet unknown genes that modify disease progression, which could be found through additional screening approaches using the fly. Importantly, there have been instances where genes discovered in *Drosophila* have been translated to HD mouse models.

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1. Huntington's disease models

Huntington's disease (HD) is an autosomal dominant inherited disease caused by a polyglutamine (polyQ) repeat expansion in the Huntingtin gene (HTT). The length of the polyQ repeat is directly proportional to the age-of-onset, however genetic variation and environmental components alter this correlation (Gusella and MacDonald, 2009). An expanded polyQ repeat leads to the accumulation of huntingtin (Htt) within cells of the CNS and causes the preferential death of medium spiny neurons (MSNs) within the striatum (DiFiglia, 1997a; DiFiglia et al., 1997b). The key to discovering novel treatments for HD is to determine the primary mechanisms that lead to this degeneration. Since human post-mortem tissue is of limited supply, a significant insight into the disease processes has been established through model systems. Both rodent and cellular models of HD provide a means by which pathophysiological processes can be investigated and manipulated in a tightly controlled manner and thus have been instrumental to pre-clinical research. However, there has also been a significant, yet perhaps less well-known, contribution to our understanding of HD using *Drosophila melanogaster* or fruit fly.

The primary means of modeling HD has been through the generation of a series of transgenic and Knock in (KI) mice. The first transgenic mouse models expressed exon 1 of the HTT gene

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containing an expanded polyQ region of between 115 and 140 repeats (Cha et al., 1998; Hansson et al., 1999). These mice display a rapid increase in Htt aggregate formation leading to the early degeneration of MSNs and a shortened life span (Bayram-Weston et al., 2012b; Brooks et al., 2012b; Mangiarini et al., 1996). The YAC128 transgenic mouse model of HD was created in 2003 and was the first to incorporate the full-length human HTT gene into its genome (Slow et al., 2003). Since this mouse model has a slower onset of phenotypes, it has been used to study prodromal phases of the disease (Bayram-Weston et al., 2012c; Brooks et al., 2012c). Similarly, the BACHD transgenic mouse model displays HD like phenotypes, which are relatively slow in progression (Gray et al., 2008). Subsequently, KI mouse models have been created to express HTT under its endogenous promoter to avoid possible overexpression artifacts. These mice have been created to express the full-length HTT gene containing various lengths of polyQ repeats, namely the Q92, Q111 and Q150 (Brooks et al., 2012a; Menalled et al., 2003; Trueman et al., 2008; Wheeler et al., 2000). These show both pathological hallmarks and behavioral traits, recapitulating the human condition (Bayram-Weston et al., 2012a,d; Brooks et al., 2012a; Menalled et al., 2003; Trueman et al., 2008, 2012; Wheeler et al., 2000). However, due to their subtle phenotypes, and minimal loss of MSNs most research has been conducted using homozygous KI mice. The most recent KI mouse model, Q175, more closely recapitulates the human disease, as multiple phenotypes can be observed in the heterozygous condition (Smith et al., 2014a).

Although these mouse models have undoubtedly been of considerable use to the HD community, experiments are often costly and long in duration. Consequently, there have also been considerable efforts to model the disease *in vitro*. Human fibroblast cultures derived from HD patients and primary neuronal cultures from transgenic mice have frequently been used as model systems. HD patient derived fibroblasts display a slower proliferation rate (Barkley et al., 1977), a decreased DNA repair capacity (Delhanty et al., 1981), show heightened L-glutamate toxicity (Gray et al., 1980), ubiquitin-proteasome system dysfunction (Seo et al., 2004) and mitochondrial abnormalities (del Hoyo et al., 2006). However since mutant Htt causes toxicity in a neuronal specific manner, key pathogenic features, such as disrupted axonal transport, cannot be measured in fibroblasts. Induced pluripotent stem cell (iPSC) derived neurons have therefore been generated from dermal fibroblasts, which display a HD like pathology when transplanted into rats (Jeon et al., 2012). Stably transfected cells expressing mutant Htt have also been used for high throughput drug screening to identify a number of compounds, which mitigate mutant Htt induced pathology (Coufal et al., 2007; Lazzeroni et al., 2013). Although HD cell lines can model certain aspects of the disease, they lack the multifactorial and age dependent components of an intact *in vivo* system.

A pertinent consideration in modeling the disease is the fact that we are not limited to mammalian model systems, since the Htt gene sequence and function is conserved from humans to invertebrate species. *Drosophila* expresses a homologue of Htt (DmHtt) that is similar in size to human Htt, distributed similarly, and shares sequence homology with human Htt at 5 different regions of the protein, including both amino terminals (Li et al., 1999). In this review we will therefore discuss the use of *Drosophila* as a model system of choice for primary HD research. Specifically, the tools available for the visualization of proteins, genetic manipulation and, importantly, how it is possible to discover new genes that are affected in HD through genetic screening approaches. There are also a number of instances where target discovery in *Drosophila* has been translated to mammalian model systems in a therapeutic context. The speed of use and tools available for this *in vivo* system make fly models an invaluable model of choice for discovery research in HD.

2. *Drosophila* models of HD

Numerous *Drosophila* models of HD have been created to investigate both degeneration and protein aggregation. Here, we discuss the phenotypes of several of these models, highlighting their relevance to HD pathology. Transgenic flies have been created to overexpress fragments of human Htt containing tracts of 2, 75, and 120 polyQ repeats (Jackson et al., 1998). These were flanked with the first 17 amino acids of Htt and an additional 135 amino acids of Htt after the polyQ repeat expansion. When expanded transgenes were expressed in the fly compound eye it caused a progressive degeneration of photoreceptor neurons (Jackson et al., 1998). Repeat length was positively correlated with both the onset and severity of the phenotype. Mutant Htt accumulation in nuclei could also be seen at 10 days post eclosion (emergence from the pupal case as an adult) (Jackson et al., 1998). Transgenic flies have also been produced to express exon 1 with either 20 or 93 polyQ repeats (Steffan et al., 2001). Pan-neuronal expression of the 93Q Htt transgene caused lethality in 70% of cases and premature death in the remaining flies compared to the expression of 20Q (Steffan et al., 2001). Transgenic flies expressing 18Q, 48Q or 152Q fused to eGFP have also been produced (Doumanis et al., 2009), yet did not develop degeneration in the compound eye. With the addition of a nuclear localization sequence, flies overexpressing 152Q::eGFP developed a subtle rough eye pigmentation, indicating degeneration. However coexpression of the 152Q::eGFP and the 48Q::eGFP transgene containing a nuclear localization sequence, caused a progressive loss of eye pigmentation observed at 21 days post eclosion (Doumanis et al., 2009). These studies highlight the conserved pathogenic function of Htt in both the cytoplasmic and nuclear compartments in *Drosophila*.

In addition to the models expressing amino-terminal fragments derived from exon 1 of human Htt, there are fly models expressing longer fragments of Htt. One model expresses a 336 amino acid fragment of human Htt containing 128Q and its expression causes an abnormal eye phenotype (Kaltenbach et al., 2007). A different model expresses a 548 amino acid fragment with 128Q, specifically designed to be a similar size as a caspase-3 cleaved Htt fragment (Lee et al., 2004). Flies expressing this fragment of Htt in the eye exhibit photoreceptor degeneration. Remaining photoreceptors were associated with a decrease in depolarization. This phenotype was not purely a developmental phenomenon, as inducing expression in adults *via* heat shock caused the same phenotype. Pan-neuronal expression of this 128Q fragment caused flies to exhibit abnormal motor and grooming behaviors and caused premature death at approximately 25 days post eclosion. The human Htt 0Q control fly had no phenotype. At the cellular level, the 548 amino acid Htt fragment caused the development of Htt-containing inclusions in the cytoplasm and neurites, but not in the nucleus (Lee et al., 2004).

A model overexpressing full-length human Htt with a 128Q repeat expansion also shows a neurodegenerative phenotype, while its 16Q control does not (Romero et al., 2008). When expressed in the eye, this full-length Htt transgene causes a progressive eye degeneration phenotype. Pan-neuronal expression of full-length 128Q Htt causes progressive motor defects, such as reduced climbing and flying performance. These flies exhibit synaptic firing defects in the neurons innervating the flight muscles and in larval neurons. The full-length transgene, however does not cause aggregation in larval neurons and is distributed throughout the cytoplasm (Romero et al., 2008). An aggregation phenotype in aged adults has not yet been investigated. While expression of various mutant Htt fragments in the eye clearly give robust phenotypes, it is also important to determine the effect of mutant Htt in specific neuronal subtypes. Of particular importance maybe to drive mutant