Chapter 1 Modelling of human polyglutamine neurological disorders in *Drosophila*

Capítulo 1 Modelado de enfermedades neurológicas por expansión de glutaminas en *Drosophila*

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Abstract

Polyglutamine (PolyQ) expansion diseases are a family of autosomal dominant neurodegenerative disorders that includes Huntington's disease and spinocerebellar ataxias. These diseases are caused by an abnormal number of glutamines repeats in the affected proteins. Different in vitro and in vivo models have been developed to study these diseases; in this review, we will focus on the fruit fly, *Drosophila melanogaster*, as a model organism to study PolyQ diseases in humans, resulting in a better understanding of PolyQ pathologies and opening avenues to potential therapeutical treatments.

Neurodegenerative disorders, *Drosophila melanogaster*, Animal models, Autosomal dominant diseases, Polyglutamine expansions

Resumen

Las enfermedades causadas por expansiones de poliglutaminas (PolyQ) son una familia de enfermedades neurodegenerativas autosómicas dominantes que incluyen la enfermedad de Huntington y las ataxias espinocerebelares. Estas enfermedades están causadas por un numero anormal de repeticiones de glutamina en las proteínas afectadas. Diferentes modelos in vitro e in vivo han sido desarrollados para estudiar estas enfermedades; en esta revisión, nos enfocaremos en la mosca de la fruta, *Drosophila melanogaster*, como modelo para el estudio de las enfermedades PolyQ en humanos, obteniendo un mejor entendimiento de estas patologías y abriendo nuevas avenidas para terapias potenciales.

Enfermedades neurodegenerativas, *Drosophila melanogaster*, Modelos animales, Enfermedades autosómicas dominantes, Enfermedades poliglutamínicas

1.1 Introduction

Polyglutamine (PolyQ) expansion diseases encompass a family of forty autosomal dominant neurodegenerative disorders that affect neurons in the cerebral cortex, basal ganglia, the cerebellum, and sometimes the retina (Shao and Diamond, 2007; Srinivasan *et al*., 2023). These disorders share symptoms like chorea, ataxia, weakness, cognitive impairment, and eye degeneration among others (Takahashi *et al*., 2010). The most widely known PolyQ disease is Huntington's Disease (HD), which affects approximately 41 000 people in the United States (Huntington's Disease Society of America, 2022), and 8000 people in Mexico (Instituto Nacional de Neurología y Neurocirugía MVS, 2018). Other PolyQ diseases are spinal bulbar muscular atrophy (SBMA), dentatorubral-pallidoluysian atrophy (DRPLA), and seven different spinocerebral ataxias (SCA) classified as 1, 2, 3, 6, 7, 8 and 17, depending on the affected protein (Shao & Diamond, 2007). SCA3, SCA2, and SCA6 are the most common ataxias worldwide; SCA17 is the rarest (Salas-Vargas *et al*., 2015; McIntosh *et al*., 2021). SCA7, although rare, has a high prevalence in Veracruz, México due to a founder effect, and it accounts for 7.4% of all SCA cases in the country (García-Velázquez *et al*., 2013; Salas-Vargas *et al*., 2015).

These pathologies are caused by an increased number of CAG (glutamine) repeats in the affected proteins; healthy proteins contain between 35–50 glutamine repeats, whereas mutated proteins contain between 40 to more than 100 repeats (Table 1.1) (Cohen-Carmon and Meshorer, 2012). These expansions are unstable and, consequently, the number of repeats changes between different cells in the same individual. These diseases also show "genetic anticipation" meaning that the number of repeats increases with every generation, and with it the onset and severity of the disease (Cohen-Carmon and Meshorer, 2012; Goswami *et al*., 2022; Srinivasan *et al*., 2023). In some cases, a non-pathological allele can expand to become pathological within a generation (McIntosh *et al*., 2021, Goswami *et al*., 2022).

Table 1.1 PolyQ expansions in human polyglutamine diseases

Disease	Protein affected	PolyQ repeat length	
		Normal	Pathogenic
Huntington´s disease (HD)	Huntingtin (HTT)	6-35	36-180
Spinal and bulbar muscular atrophy (SBMA)	Androgen receptor (AR)	9-36	38-65
Dentatorubral-pallidoluysian atrophy (DRPLA)	Atrophin 1 (ATN1)	6-36	49-80
Spinocerebellar ataxia type 1 (SCA1)	Ataxin 1 (ATXN1)	6-39	39-83
Spinocerebellar ataxia type 2 (SCA2)	Ataxin 2 (ATXN2)	14-32	32-200
Spinocerebellar ataxia type 3 (SCA3)	Ataxin 3 (ATXN3)	12-41	55-84
Spinocerebellar ataxia type 6 (SCA6)	Calcium channel α1A subunit (CACNA1A)	4-19	20-33
Spinocerebellar ataxia type 7 (SCA7)	Ataxin 7 (ATXN7)	4-35	37-306
Spinocerebellar ataxia type 8 (SCA8)	Ataxin 8 (ATXN8)	15-50	54-250
Spinocerebellar ataxia type 17 (SCA17)	TATA-binding protein (TBP)	25-44	46-63

The molecular mechanism behind PolyQ diseases is not completely understood, but it is clear that the PolyQ regions are the common denominator for the diseases, given that the proteins involved differ in sequence, structure, and biological function (McIntosh *et al*., 2021). It is thought that, just like in the case of Alzheimer's Disease and Parkinson's Disease, protein aggregates might play a role in the toxicity that causes development of neurodegeneration (Takahashi *et al*., 2010). In this case, the conformational changes brought on by the PolyQ extensions would cause misfolded proteins to combine, and in turn, these aggregates would either sequester proteins, leading to a loss of function, or interfere with nuclear or neuronal function (Takahashi *et al*., 2010; McIntosh *et al*., 2021). Takahashi and colleagues (2010) claim that aggregation alone does not explain the pathological features of PolyQ diseases, but rather the interaction between mutated proteins with other proteins in the different neurons affected.

In Huntington's disease, the mutant huntingtin protein (mHTT) has an abnormally expanded polyglutamine repeat. Mutant huntingtin causes neuronal dysfunction and death due to aggregates that cause cell toxicity and interfere with neural processes like axonal transport, transcription, translation, and synaptic function. Medium spiny neurons (MSNs) of the striatum are the most affected by mHTT, resulting in the clinical marks of this disease like motor disturbances with prominent chorea in the early stages. Cognitive issues may present many years before symptoms start to show, and they are characterized by impaired visuospatial and executive function, processing speed, and emotion recognition (McColgan and Tabrizi 2017). It was recently reported that HD also affects peripheral tissues via a crosstalk with the nervous system (Gómez-Jaramillo, 2022)

Spinal and bulbar muscular atrophy (SBMA) is a rare hereditary lower motor neuron disease characterized by progressive muscular weakness. This was the first evidence of a pathogenic expanded trinucleotide repeat causing a disease. The mutation responsible occurs in the androgen receptor gene (AR) with an expanded trinucleotide repeat (CAG > 37). In SBMA, toxicity is caused by the formation of nuclear inclusions of the mutant receptor. This impairs AR function, affecting both motor neurons and muscles, and causing endocrine manifestations such as gynecomastia and infertility (Breza and Koutsis, 2018).

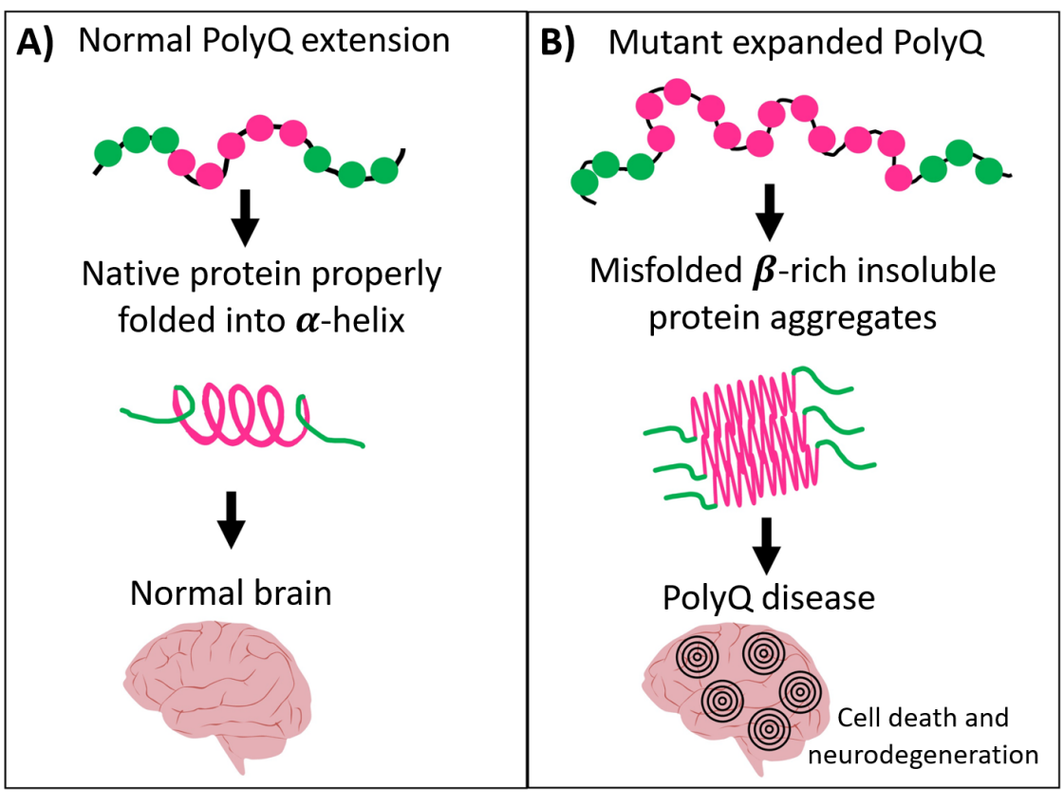
Dentatorubral-pallidoluysian atrophy (DRPLA) is an autosomal dominant hereditary ataxia caused by mutation in the Atrophin-1 (ATN1) gene with a CAG trinucleotide repeat expansion (≥48 tandem copies). DRPLA symptoms include epilepsy, ataxia, choreoathetosis, and dementia. Clinical presentation varies: in younger people, the disease is often characterized by seizures, and it more commonly presents with ataxia and cognitive impairment in older patients. Clinical symptoms are progressive, with life expectancy typically 8–16 years (Tsuji, 2012).

Spinocerebellar ataxias (SCAs) are a genetically heterogeneous conditions that can be inherited, and are autosomal dominant or recessive (Orr, 2012; Paulson *et al*., 2017). Patients present a cerebellar syndrome marked by loss of balance and coordination accompanied by cortical symptoms (seizures, cognitive impairment), peripheral neuropathy and slurred speech; onset most often occurs in adult life (Manto and Marmolino, 2009). SCA mutations cause cerebellar atrophy by prominent damage to Purkinje neurons. About 30 of these conditions have been described, however, only seven —SCA1, SCA2, SCA3, SCA6, SCA7, SCA8 and SCA17—are caused by an expanded polyglutamine region in the protein affected. The PolyQ proteins tend to accumulate in the nucleus or cytoplasm of cells, affecting gene expression, organization and cell function, and other functions. Some mutant PolyQ proteins responsible for SCAs are ataxin-1, -2, -3, -7, and -8 (SCA1, SCA2, SCA3, SCA7, and SCA8), the α1A subunit of the calcium channel CACNA1A (SCA6), and the TATA-binding protein (TBP) in SCA17 (Orr and Zoghbi, 2007; Chongtham and Agrawal, 2016).

1.2 Structure of PolyQ aggregates

The conformation of the PolyQ expanded aggregates was discovered in 1994 by Perutz and collaborators; they reported that chemically synthesized expanded PolyQs, under normal physiological conditions, form rich aggregates of properly folded a-sheet structures (Figure 1.1). These a-sheet-rich monomers are preceded by a helical conformation and followed by a random coil that forms soluble oligomers. In the expanded protein, the PolyQ stretch folds into β-sheet-rich monomers that form soluble oligomers that aggregate in an insoluble amyloid-like structure (Perutz *et al*., 1994; Minakawa and Nagai 2021; Mier & Andrade-Navarro 2021). Normally, PolyQ fibers with a single, 20-glutamine helical twist are unstable and therefore removed, but a double stretch of 40 glutamines is held together by polar zippers formed by hydrogen bonds between amides of successive turns (Perutz *et al*., 1994). It has been described that α-helical coiled-coil structures also contribute to PolyQ protein toxicity (Fiumara *et al*., 2010: Kwon *et al*., 2018). It is not yet clear whether the aggregates adopt this conformation before or after polymerization, that is, whether the transition to a β-sheet conformation causes the PolyQ oligomerization or whether PolyQ oligomerization causes the conformational change (Hoffner and DJian, 2015).

Figure 1.1 Expanded PolyQ aggregation and toxicity. A) Schematic representation of a protein containing a normal PolyQ extension, which is properly folded into an α-helix to perform its cellular functions in the brain. B) A disease-causing protein with an expanded PolyQ region undergoes aberrant folding from an α-helix-rich structure into a β-sheet-rich conformation state, followed by the formation of insoluble aggregates with amyloid-like structures. The insoluble aggregates lead to neural cell toxicity, and eventually neurodegeneration



Different microscopy techniques have been used to elucidate additional structures that could be present in these protein aggregates; this may contribute to describe not only structure or toxicity features, but also how the aggregates are formed within cells (Legleiter *et al*., 2010; Nucifora *et al*., 2012; Olshina *et al*., 2010).

PolyQ diseases are being studied with in vitro and in vivo models. Microarrays and fluorescence resonance energy transfer (FRET) have been used to study gene expression and the interactions of mutated proteins in living cells, respectively (Luthi-Carter *et al*., 2002; Takahashi *et al*., 2010). Pluripotent stem cells are also a promising model to study these diseases (Cohen-Carmon & Mesroher, 2012; Naphade *et al*., 2019). Transgenic mice have been used to study spinocerebellar ataxias (Burright *et al*., 1995; Bichelmeier *et al*., 2007; Watase, 2014; Meierhofer *et al*., 2016) and Huntington's disease (Stack *et al*., 2005, Brooks & Dunnett, 2013, Yang *et al*., 2017, Dunnett & Brooks, 2018, Farshim & Bates, 2018, Kosior & Leavitt, 2018, Back *et al*., 2021). The fruit fly, *Drosophila melanogaster*, has also been used as a model to replicate the phenotypic characteristics of PolyQ diseases (Koon & Chan, 2017; Rosas-Arellano *et al*., 2018,)

Model organisms have greatly increased our understanding of these pathologies, and in the development of potential treatments. In this review, we will focus on the fruit fly, *Drosophila melanogaster*, as an idoneous model organism for neurodegenerative diseases.

1.3 *Drosophila* as a model for neurodegenerative diseases

In 1900, entomologist Charles W. Woodworth proposed using *Drosophila melanogaster* as a genetic model organism. Nine years later, Prof. Morgan established his lab for genetic experiments using the fruit fly (Sturtevant, 1959). Since then, hundreds of studies have been carried out with *D. melanogaster*, consolidating it as a model organism and expanding the knowledge in crucial matters such as genes, chromosomes, and inheritance of genetic information. Its success as a model organism also relies on its small size, easy husbandry, and inexpensive maintenance and manipulation in the laboratory: *D. melanogaster* has a rapid life cycle and produces large amounts of genetically identical progeny in 10 days at 25 °C, generating large amounts of data for statistical analyses. Another valuable characteristic is its external development which facilitates visualization (Yamaguchi & Yoshida, 2018).

Genes between fruit flies and humans are highly conserved, moreover, the similarity does not stop at the genetic level: *D. melanogaster* possesses a complex nervous system and behaviors including social activity, learning and memory. Additionally, about 75% of the genes involved in human diseases have their homologue in *D. melanogaster* (Yamamoto *et al*., 2014), making it an excellent prospect for studying neuronal dysfunction and neuronal death derived from various neurodegenerative diseases (Chan & Bonini, 2000).

Some neurodegenerative disorders studied in *Drosophila* include Alzheimer, Parkinson, tauopathies, ALS, prions (PrD), dystonia, noncoding expansions (SCA8, myotonic dystrophy), some recessive disorders including fragile X syndrome and Friedreich's ataxia, and various polyglutamine disorders (Huntington disease, SCA1, SCA3, and spinobulbar muscular atrophy) (Xu *et al*., 2015; Tzou *et al*., 2022).

Modeling of human neurodegenerative diseases in *D. melanogaster* is achieved through the "humanization" of the fly, which is modified using the UAS/GAL4 binary system (Brand & Perrimon, 1993) to express the pathogenic human protein related to the disease in question (Marsh & Thompson, 2006). Once "humanized", *D. melanogaster* offers multiple advantages to study the molecular and cellular mechanisms involved in the disease to be studied: well-known anatomy, well-characterized gene promoters, and a wide variety of mutants (Venken & Bellen, 2005). In the next section, we will describe some *Drosophila* models for PolyQ diseases

1.4 *Drosophila* as a model for PolyQ diseases

The first PolyQ diseases modeled in *D. melanogaster* were SCA1 and SCA3 (Warrick *et al*., 1998; Fernandez-Funez *et al*., 2000). The modeling of SCA1 in *D. melanogaster* elucidated the genetic and molecular mechanisms underlying neuronal degeneration by expressing the human SCA1 gene in *Drosophila*. According to the experiments, high levels of wild-type ATXN1 were found to cause degenerative phenotypes like those caused by expanded PolyQ proteins. In addition, the research team corroborated that flies that expressed high-levels of wild-type ATXN1 shared the same toxic effects of the ones expressing the ATXN1 protein with an expanded PolyQ region. This was patent in the negative geotaxis assay, were both cohorts of flies were unable to fly as high as the flies in the control group (Fernandez-Funez *et al*., 2000). Recently, by performing a cross-species genetic screening, a total of 22 mutant regulators of ATXN1 were described in *Drosophila melanogaster*. Among them, transglutaminase 5 (TG5) stood out; in this case, TG5 preferentially regulated mutant ATXN1 over the wild-type protein. In physiological conditions, TG enzymes catalyze the cross-linking of ATXN1 in a polyQ-length-dependent manner, modulating mutant ATXN1 stability and oligomerization. When Tg expression was silenced in a *Drosophila* SCA1 model, mutant ATXN1 toxicity was modulated (Lee *et al*., 2022).

Another *D. melanogaster* model was created for SCA3, where only a segment of the ATXN3 protein containing the expanded polyglutamine region was expressed in the flies. This time, however, the protein was expressed in the photoreceptor neurons. This experiment allowed the researchers to observe the degeneration of the eye tissues in the fruit fly, which indicated cell degeneration. The most affected fly was the one expressing the protein with 78 glutamines, which presented abnormally thin and severely depigmented eyes, due to severe loss of eye cells (Xu *et al*., 2015). Another possible mechanism involved in SCA3 pathogenesis was thought to be non-AUG translation or also known as RAN translation which has been documented in polyglutamine (polyQ) disorders. However, when studied in a SCA3/MJD *Drosophila* model, there was no unconventional translation in fly neurons or glia (Johnson *et al*., 2022).

Another model for SCA7 was attempted in *D. melanogaster* but the expanded PolyQ ATXN7 protein remained stable regardless of the context in which it was expressed. Consequently, it was posited that, during evolution, selective pressure allowed *D. melanogaster* to develop robust mechanisms to maintain PolyQs within a controlled range, which has not been observed in mammals (Jackson *et al*., 2005).

Additionally, Jun Ma's group established a model of SCA17 in *Drosophila*, in which hTBP34, 54 and 80Q was expressed in the eye. Transgenic flies expressing a mutant hTBP protein with an expanded PolyQ tract (hTBP80Q) show progressive degeneration in the ommatidia of the eye, a characteristic that was extrapolated with the neurodegeneration present in the brains of SCA17 patients. The authors focused mainly on gene expression, which they measured with PolyQ, found deregulations in transcription that suggested that the activity of the transcription factor Su(H) is involved in the pathological progression in SCA17 patients (Ren *et al*., 2011).

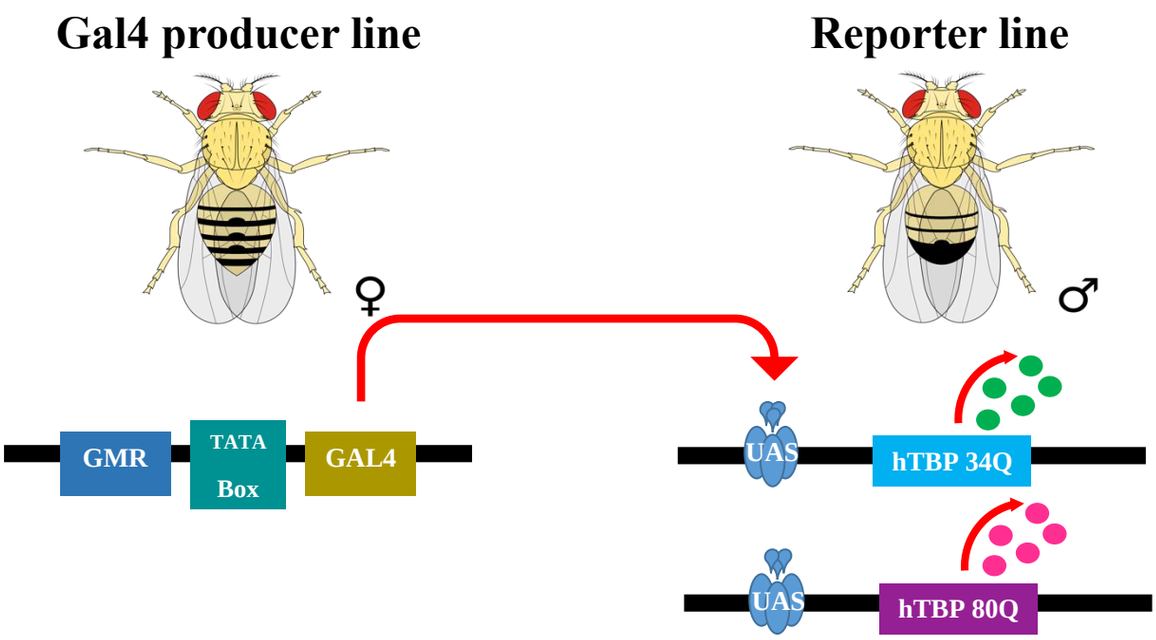
SMBA has also been modeled in *D. melanogaster*, where it was observed that the androgen receptor (AR) protein with the expanded PolyQ region can translocate to the nucleus and activate AR-dependent pathway transcription. *Drosophila melanogaster* loses motor neurons when the expanded PolyQ protein is expressed, hence, showing loss of climbing ability and affected gait (Nedelsky et al., 2010).

Last but not least, Huntington’s disease PolyQ has also been modeled in the fruit fly. This model displayed the same phenotypes as the other PolyQ: the degeneration of the eye and loss of climbing ability. Fly lines with the expanded PolyQs HD-Q75 and HD-Q120 had normal eye morphology and intact ommatids on hatching day (day 0), but by day 10, many rhabdomeres were disrupted, with more obvious and severe degeneration in HD-Q120 flies than in HD-Q75 (Zhang et al., 2010).

1.5 Humanizing the fly

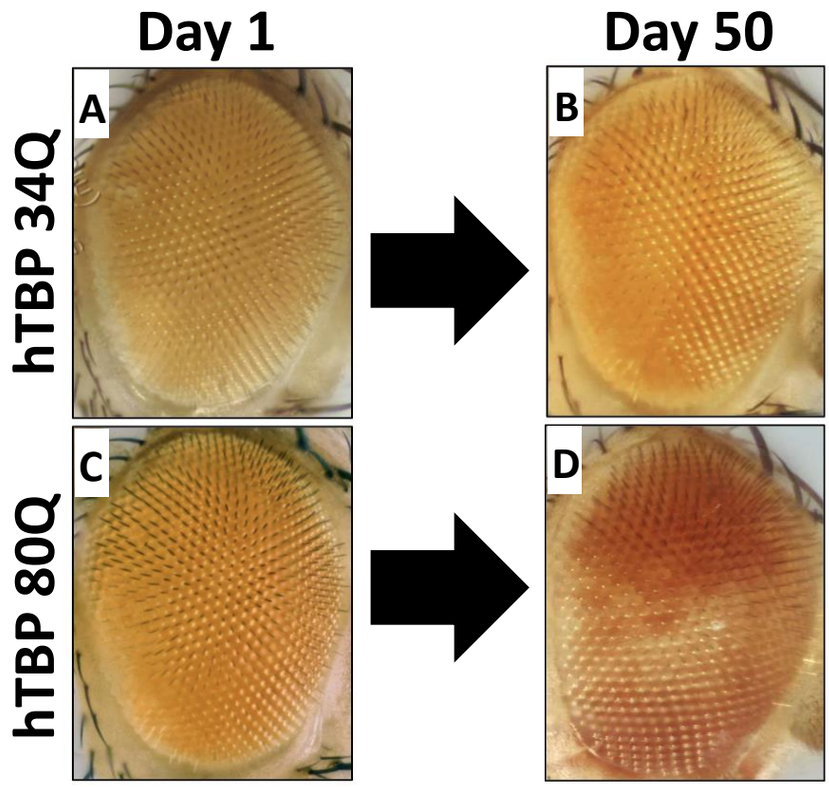
The “humanization” of flies—their capacity to express human proteins—is possible thanks to a system called UAS-GAL4. Andrea Brand and Norbert Perrimon standardized this method in flies in 1993 and it has been used to study the expression of genes ever since. The system consists of crossing a GAL4 producer line or driver and a UAS reporter line. The producer line encodes and expresses the GAL4 trans-activator protein under the control of a tissue-specific promoter or enhancer; the reporter line contains the gene encoding the protein of interest under the control of UAS (Upstream Activator Sequence) sequences where GAL4 binds to activate transcription (Fig. 1.2). When crossing both flies’ lines, the progeny that contains both transgenes, in a tissue-specific manner, will express the GAL4 protein which in turn will bind to the UAS sequence and promote the transcription of the gene of interest only in the regions of the promoter’s specificity.

Figure 1.2 Expression of human TBP gene through the UAS/GAL4 binary system. The GAL4 gene encodes a trans-activator protein introduced into *D. melanogaster* genome and its expression is regulated by a tissue-specific promoter in the producer line that will express GAL4 in specific tissues and cells. In this case, because GMR is the promoter, GAL4 will be expressed in the eye neurons of *Drosophila melanogaster*, where it will bind to the UAS sequences and allow the expression of hTBP 34Q or hTBP 80Q



In our laboratory, we modeled SCA17 in *D. melanogaster* to determine the cytotoxic effect of elongated glutamines in the TATA binding-protein (TBP). Using the UAS-Gal4 system (Fig. 1.2), we generated flies that, in their eyes, expressed human TBP with 34 (hTBP 34Q) and 80 (hTBP 80Q) glutamines and evaluated them for 50 days. We found that, in the flies with hTBP 80Q, the eyes degenerated more as the fly aged, as shown by eye color decrease and sometimes tissue disorganization (Fig. 1.3) (Cárdenas-Tueme, 2017).

Figure 1.3 Expression of hTBP34Q and hTBP80Q targeting the eyes of *Drosophila melanogaster*. Brightfield micrographs of the *Drosophila* eye show that GMR-directed expression of hTBP 34Q and hTBP 80Q causes impairments in the fruit fly eye. (A-B) Expression of hTBP 34Q at different times: Day 1 and 50 days after hatching, the phenotype caused by hTBP 34Q is not aggressive, a slight persistent discoloration is observed throughout the days in the perimeter of the eye. (C-D) Expression of hTBP 80Q at 1 and 50 days after hatching. (C) On day 1, depigmentation is seen around the eye's perimeter. (D) The discoloration has spread towards the center of the eye at day 50, so the hTBP 80Q protein appears to be more toxic



1.6 Drug screening in *Drosophila melanogaster*

Drug development has high failure rates, is extremely expensive (Wouters *et al*., 2018), and usually encompasses a long design and testing process. Alternative approaches for drug discovery, such as the use of *D. melanogaster* as a model to test new drugs, would greatly benefit the pharmaceutical industry.

For instance, the efficacy of diverse drugs or chemical compounds against neurological diseases can be studied in *Drosophila* (Lawal *et al*., 2014). Humanized *Drosophila* may help to determine the adequacy of a drug at an early stage before it is tested in more expensive rodent assays and in clinical trials.

The easiest and most common way to do this is to keep 10-15 flies in a vial and add the chemical or drug directly to the food where the flies are kept. The flies will be monitored for several days, and it will be documented whether the flies exposed to the chemical/drug have a shorter life expectancy than those that were not exposed. Behavioral changes, such as flying, can also be assessed. Furthermore, thanks to the genome structure of the fly and its reduced genetic redundancy, the molecular mechanism of drug action will be easier to elucidate.

Even though *Drosophila* holds promise for drug discovery, there are limitations, particularly related to toxicity and pharmacodynamics that deserve attention, however this can be resolved sooner rather than later to benefit from this model organism.

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1.8 Funding

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1.9 Conclusions

Neurodegenerative diseases, including the polyQ disorders such as Huntington disease and spinocerebellar ataxias share the feature of abnormal protein accumulation. Here we contend that *Drosophila* is a promising model of polyQ diseases including HD, SCA1, SCA3 and SCA17 in the study of the pathological effects of polyQ expansions. In our lab, we created a SCA17 model in *Drosophila* using an expansion of a polyQ repeat in the TBP transcriptional factor. The transgenic flies expressing an expanded polyQ hTBP protein exhibited progressive neurodegeneration very similar to SCA17 patients. The use of animal models, such as *Drosophila* may also open new therapeutic avenues for the diagnosis and treatment of PolyQ diseases.

Since polyQ diseases remain uncurable, modeling of these diseases in organisms such as *Drosophila* presents great potential and advantages to examine numerous therapeutic strategies effective against polyQ diseases. Humanizing the fly allows to test pharmacological and biological molecules to block the early events of the aggregation process and inclusions, as well as degradation of toxic proteins and regulation of cellular function.

1.10 References

Back, M. K., Kurzawa, J., Ruggieri, S., & von Engelhardt, J. (2021). C57BL/6 Background Attenuates mHTT Toxicity in the Striatum of YAC128 Mice. *International Journal of Molecular Sciences*, *22*(23), 12664. doi:10.3390/ijms222312664 URL: www.mdpi.com/1422-0067/22/23/12664

Bichelmeier, U., Schmidt, T., Hubener, J., Boy, J., Ruttiger, L., Habig, K., Poths, S., Bonin, M., Knipper, M., Schmidt, W. J., Wilbertz, J., Wolburg, H., Laccone, F., & Riess, O. (2007). Nuclear Localization of Ataxin-3 Is Required for the Manifestation of Symptoms in SCA3: In Vivo Evidence. *Journal of Neuroscience*, *27*(28), 7418–7428. doi:10.1523/jneurosci.4540-06.2007 URL: https://www.jneurosci.org/content/27/28/7418

Brand, A., & Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development*, *118*(2), 401–415. doi:10.1242/dev.118.2.401 URL: https://journals.biologists.com/dev/article/118/2/401/37970/Targeted-gene-expression-as-a-means-of-altering

Breza, M., & Koutsis, G. (2018). Kennedy's disease (spinal and bulbar muscular atrophy): a clinically oriented review of a rare disease. *Journal of Neurology*, *266*(3), 565–573. doi:10.1007/s00415-018-8968-7 URL: https://link.springer.com/article/10.1007/s00415-018-8968-7

Brooks, S. P., & Dunnett, S. B. (2013). Mouse Models of Huntington's Disease. *Behavioral Neurobiology of Huntington's Disease and Parkinson's Disease*, 101–133. doi:10.1007/7854_2013_256 URL: https://link.springer.com/chapter/10.1007/7854_2013_256

Burright, E. N., Brent Clark, H., Servadio, A., Matilla, T., Feddersen, R. M., Yunis, W. S., Duvick, L. A., Zoghbi, H. Y., & Orr, H. T. (1995). SCA1 transgenic mice: A model for neurodegeneration caused by an expanded CAG trinucleotide repeat. *Cell*, *82*(6), 937–948. doi:10.1016/0092-8674(95)90273-2 URL: https://www.cell.com/cell/pdf/0092-8674(95)90273-2.pdf?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2F0092867495902732%3Fshowall%3Dtrue

Cárdenas-Tueme, M. (2017). *Efecto patológico de la expansión de poliglutaminas de hTBP en la enfermedad neurodegenerativa SCA17 en* Drosophila melanogaster. [Tesis de Maestría, Universidad Autónoma de Nuevo León] Repositorio Académico Digital UANL http://eprints.uanl.mx/17690/

Chan, H. Y. E., & Bonini, N. M. (2000). *Drosophila* models of human neurodegenerative disease. *Cell Death & Differentiation*, *7*(11), 1075–1080. doi:10.1038/sj.cdd.4400757 URL: https://www.nature.com/articles/4400757

Chongtham, A., & Agrawal, N. (2016). Curcumin modulates cell death and is protective in Huntington's disease model. *Scientific Reports*, *6*(1). doi:10.1038/srep18736 URL: https://www.nature.com/articles/srep18736

Cohen-Carmon, D., & Meshorer, E. (2012). Polyglutamine (polyQ) disorders. *Nucleus*, *3*(5), 433–441. doi:10.4161/nucl.21481 URL: https://www.tandfonline.com/doi/full/10.4161/nucl.21481

Dunnett, S. B., & Brooks, S. P. (2018). Motor Assessment in Huntington's Disease Mice. *Methods in Molecular Biology*, 121–141. doi:10.1007/978-1-4939-7825-0_7 URL: https://link.springer.com/protocol/10.1007/978-1-4939-7825-0_7

Farshim, P. P., & Bates, G. P. (2018). Mouse Models of Huntington's Disease. *Methods in Molecular Biology*, 97–120. doi:10.1007/978-1-4939-7825-0_6 URL: https://link.springer.com/protocol/10.1007/978-1-4939-7825-0_6

Fernandez-Funez, P., Nino-Rosales, M. L., de Gouyon, B., She, W. C., Luchak, J. M., Martinez, P., Turiegano, E., Benito, J., Capovilla, M., Skinner, P. J., McCall, A., Canal, I., Orr, H. T., Zoghbi, H. Y., & Botas, J. (2000). Identification of genes that modify ataxin-1-induced neurodegeneration. *Nature*, *408*(6808), 101–106. doi:10.1038/35040584 URL: https://www.nature.com/articles/35040584

Fiumara, F., Fioriti, L., Kandel, E. R., & Hendrickson, W. A. (2010). Essential Role of Coiled Coils for Aggregation and Activity of Q/N-Rich Prions and PolyQ Proteins. *Cell*, *143*(7), 1121–1135. doi:10.1016/j.cell.2010.11.042 URL: https://www.sciencedirect.com/science/article/pii/S0092867410013644?via%3Dihub

García-Velázquez, L. E., Canizales-Quinteros, S., Romero-Hidalgo, S., Ochoa-Morales, A., Martínez-Ruano, L., Márquez-Luna, C., Acuña-Alonzo, V., Villarreal-Molina, M. T., Alonso-Vilatela, M. E., & Yescas-Gómez, P. (2013). Founder effect and ancestral origin of the spinocerebellar ataxia type 7 (SCA7) mutation in Mexican families. *Neurogenetics*, *15*(1), 13–17. doi:10.1007/s10048-013-0387-4 URL: https://link.springer.com/article/10.1007/s10048-013-0387-4

Gómez-Jaramillo, L., Cano-Cano, F., González-Montelongo, M. D. C., Campos-Caro, A., Aguilar-Diosdado, M., & Arroba, A. I. (2022). A New Perspective on Huntington's Disease: How a Neurological Disorder Influences the Peripheral Tissues. International Journal of Molecular Sciences, 23(11), 6089. doi: 10.3390/ijms23116089 URL: https://www.mdpi.com/1422-0067/23/11/6089

Goswami, R., Bello, A. I., Bean, J., Costanzo, K. M., Omer, B., Cornelio-Parra, D., Odah, R., Ahluwalia, A., Allan, S. K., Nguyen, N., Shores, T., Aziz, N. A., & Mohan, R. D. (2022). The Molecular Basis of Spinocerebellar Ataxia Type 7. *Frontiers in Neuroscience*, *16*. doi:10.3389/fnins.2022.818757 URL: https://www.frontiersin.org/articles/10.3389/fnins.2022.818757/full

Hoffner, G., & Djian, P. (2015). Polyglutamine aggregation in Huntington disease: does structure determine toxicity?. *Molecular neurobiology*, *52*(3), 1297-1314. doi:10.1007/s12035-014-8932-1. https://link.springer.com/article/10.1007/s12035-014-8932-1

Huntington's Disease Society of America. (2022). *Overview of Huntington's Disease Huntington's Disease Society of America*. Hdsa.Org. Retrieved May 17, 2022, from https://hdsa.org/what-is-hd/overview-of-huntingtons-disease/

Instituto Nacional de Neurología y Neurocirugía MVS. (2018, March 14). *La Enfermedad de Huntington*. Http://Www.Innn.Salud.Gob.Mx/. Retrieved April 24, 2022, from http://www.innn.salud.gob.mx/interna/medica/padecimientos/huntington.html

Jackson, S. M., Whitworth, A. J., Greene, J. C., Libby, R. T., Baccam, S. L., Pallanck, L. J., & La Spada, A. R. (2005). A SCA7 CAG/CTG repeat expansion is stable in *Drosophila melanogaster* despite modulation of genomic context and gene dosage. Gene, 347(1), 35–41. doi:10.1016/j.gene.2004.12.008 URL: https://www.sciencedirect.com/science/article/abs/pii/S0378111904007383?via%3Dihub

Johnson, S. L., Prifti, M. V., Sujkowski, A., Libohova, K., Blount, J. R., Hong, L., Tsou, W. L., & Todi, S. V. (2022). *Drosophila* as a Model of Unconventional Translation in Spinocerebellar Ataxia Type 3. Cells, 11(7), 1223. doi:10.3390/cells11071223 URL: https://www.mdpi.com/2073-4409/11/7/1223

Koon, A. C., & Chan, H. Y. E. (2017). *Drosophila melanogaster* As a Model Organism to Study RNA Toxicity of Repeat Expansion-Associated Neurodegenerative and Neuromuscular Diseases. *Frontiers in Cellular Neuroscience*, *11*. doi:10.3389/fncel.2017.00070 URL: https://www.frontiersin.org/articles/10.3389/fncel.2017.00070/full

Kosior, N., & Leavitt, B. R. (2018). Murine Models of Huntington's Disease for Evaluating Therapeutics. *Methods in Molecular Biology*, 179–207. doi:10.1007/978-1-4939-7825-0_10 URL: https://link.springer.com/protocol/10.1007/978-1-4939-7825-0_10

Kwon, M. J., Han, M. H., Bagley, J. A., Hyeon, D. Y., Ko, B. S., Lee, Y. M., Cha, I. J., Kim, S. Y., Kim, D. Y., Kim, H. M., Hwang, D., Lee, S. B., & Jan, Y. N. (2018). Coiled-coil structure-dependent interactions between polyQ proteins and Foxo lead to dendrite pathology and behavioral defects. *Proceedings of the National Academy of Sciences*, *115*(45). doi:10.1073/pnas.1807206115 URL: https://www.pnas.org/doi/full/10.1073/pnas.1807206115

Lawal, H. O., Terrell, A., Lam, H. A., Djapri, C., Jang, J., Hadi, R., Roberts, L., Shahi, V., Chou, M. T., Biedermann, T., Huang, B., Lawless, G. M., Maidment, N. T., & Krantz, D. E. (2014). *Drosophila* modifier screens to identify novel neuropsychiatric drugs including aminergic agents for the possible treatment of Parkinson's disease and depression. *Molecular Psychiatry*, *19*(2), 235–242. doi:10.1038/mp.2012.170 URL: https://www.nature.com/articles/mp2012170

Lee, W. S., Al-Ramahi, I., Jeong, H. H., Jang, Y., Lin, T., Adamski, C. J., Lavery, L. A., Rath, S., Richman, R., Bondar, V. V., Alcala, E., Revelli, J. P., Orr, H. T., Liu, Z., Botas, J., & Zoghbi, H. Y. (2022). Cross-species genetic screens identify transglutaminase 5 as a regulator of polyglutamine-expanded ataxin-1. The Journal of clinical investigation, 132(9), e156616. doi:10.1172/JCI156616. URL: https://www.jci.org/articles/view/156616

Legleiter, J., Mitchell, E., Lotz, G. P., Sapp, E., Ng, C., DiFiglia, M., Thompson, L. M., & Muchowski, P. J. (2010). Mutant huntingtin fragments form oligomers in a polyglutamine length-dependent manner in vitro and in vivo. Journal of Biological Chemistry, 285(19), 14777-14790. doi:10.1074/jbc.M109.093708 URL: https://www.jbc.org/article/S0021-9258(20)54925-9/fulltext

Luthi-Carter, R. (2002). Dysregulation of gene expression in the R6/2 model of polyglutamine disease: parallel changes in muscle and brain. *Human Molecular Genetics*, *11*(17), 1911–1926. doi:10.1093/hmg/11.17.1911 URL: https://academic.oup.com/hmg/article/11/17/1911/589985

Manto, M., & Marmolino, D. (2009). Cerebellar ataxias. *Current opinion in neurology*, *22*(4), 419-429. doi:10.1097/WCO.0b013e32832b9897 URL:https://journals.lww.com/co-neurology/Fulltext/2009/08000/Cerebellar_ataxias.14.aspx

Marsh, J. L., & Thompson, L. M. (2006). *Drosophila* in the Study of Neurodegenerative Disease. *Neuron*, *52*(1), 169–178. doi:10.1016/j.neuron.2006.09.025 URL: https://www.sciencedirect.com/science/article/pii/S089662730600732X?via%3Dihub

McColgan, P., & Tabrizi, S. J. (2017). Huntington's disease: a clinical review. *European Journal of Neurology*, *25*(1), 24–34. doi:10.1111/ene.13413 URL: https://onlinelibrary.wiley.com/doi/10.1111/ene.13413

McIntosh, C. S., Li, D., Wilton, S. D., & Aung-Htut, M. T. (2021). Polyglutamine Ataxias: Our Current Molecular Understanding and What the Future Holds for Antisense Therapies. *Biomedicines*, *9*(11), 1499. doi:10.3390/biomedicines9111499 URL: https://www.mdpi.com/2227-9059/9/11/1499

Meierhofer, D., Halbach, M., ŞEn, N. E., Gispert, S., & Auburger, G. (2016). Ataxin-2 (Atxn2)-Knock-Out Mice Show Branched Chain Amino Acids and Fatty Acids Pathway Alterations. *Molecular & Cellular Proteomics*, *15*(5), 1728–1739. doi:10.1074/mcp.m115.056770 URL: https://www.sciencedirect.com/science/article/pii/S1535947620336094?via%3Dihub

Mier, P., & Andrade-Navarro, M. A. (2021). Between Interactions and Aggregates: The PolyQ Balance. *Genome Biology and Evolution*. doi:10.1093/gbe/evab246 URL: https://academic.oup.com/gbe/article/13/11/evab246/6427629

Minakawa, E. N., & Nagai, Y. (2021). Protein Aggregation Inhibitors as Disease-Modifying Therapies for Polyglutamine Diseases. *Frontiers in Neuroscience*, *15*. doi:10.3389/fnins.2021.621996 URL: https://www.frontiersin.org/articles/10.3389/fnins.2021.621996/full

Naphade, S., Tshilenge, K. T., & Ellerby, L. M. (2019). Modeling Polyglutamine Expansion Diseases with Induced Pluripotent Stem Cells. *Neurotherapeutics*, *16*(4), 979–998. doi:10.1007/s13311-019-00810-8 URL: https://link.springer.com/article/10.1007/s13311-019-00810-8

Nedelsky, N. B., Pennuto, M., Smith, R. B., Palazzolo, I., Moore, J., Nie, Z., Neale, G., & Taylor, J. P. (2010). Native Functions of the Androgen Receptor Are Essential to Pathogenesis in a *Drosophila* Model of Spinobulbar Muscular Atrophy. *Neuron*, *67*(6), 936–952. doi:10.1016/j.neuron.2010.08.034 URL: https://www.sciencedirect.com/science/article/pii/S089662731000677X?via%3Dihub

Muchowski, P. J., Finkbeiner, S., Legleiter, J., Ross, C. A., & Poirier, M. A. (2012). Identification of novel potentially toxic oligomers formed in vitro from mammalian-derived expanded huntingtin exon-1 protein. *Journal of Biological Chemistry*, *287*(19), 16017-16028. doi:10.1074/jbc.M111.252577 URL:https://www.jbc.org/article/S0021-9258(20)46248-9/fulltext

Olshina, M. A., Angley, L. M., Ramdzan, Y. M., Tang, J., Bailey, M. F., Hill, A. F., & Hatters, D. M. (2010). Tracking mutant huntingtin aggregation kinetics in cells reveals three major populations that include an invariant oligomer pool. *Journal of Biological Chemistry*, *285*(28), 21807-21816. doi:10.1074/jbc.M109.084434 URL:https://www.jbc.org/article/S0021-9258(20)60206-X/fulltext

Orr, H. T. (2012). Cell biology of spinocerebellar ataxia. *Journal of Cell Biology*, *197*(2), 167-177. doi:.10.1083/jcb.201105092 URL:https://rupress.org/jcb/article/197/2/167/54651/Cell-biology-of-spinocerebellar-ataxiaCell-biology

Orr, H. T., & Zoghbi, H. Y. (2007). Trinucleotide Repeat Disorders. *Annual Review of Neuroscience*, *30*(1), 575–621. doi:10.1146/annurev.neuro.29.051605.113042 URL: https://www.annualreviews.org/doi/10.1146/annurev.neuro.29.051605.113042

Paulson, H. L., Shakkottai, V. G., Clark, H. B., & Orr, H. T. (2017). Polyglutamine spinocerebellar ataxias—from genes to potential treatments. *Nature Reviews Neuroscience*, *18*(10), 613-626. doi:10.1038/nrn.2017.92 URL: https://www.nature.com/articles/nrn.2017.92

Perutz, M. F., Johnson, T., Suzuki, M., & Finch, J. T. (1994). Glutamine repeats as polar zippers: their possible role in inherited neurodegenerative diseases. *Proceedings of the National Academy of Sciences*, *91*(12), 5355-5358. doi:10.1073/pnas.91.12.5355 URL:https://www.pnas.org/doi/abs/10.1073/pnas.91.12.5355

Ren, J., Jegga, A. G., Zhang, M., Deng, J., Liu, J., Gordon, C. B., Aronow, B. J., Lu, L. J., Zhang, B., & Ma, J. (2011). A *Drosophila* model of the neurodegenerative disease SCA17 reveals a role of RBP-J/Su(H) in modulating the pathological outcome. *Human Molecular Genetics*, *20*(17), 3424–3436. doi:10.1093/hmg/ddr251 URL: https://academic.oup.com/hmg/article/20/17/3424/2527117

Rosas-Arellano, A., Estrada-Mondragón, A., Piña, R., Mantellero, C., & Castro, M. (2018). The Tiny *Drosophila melanogaster* for the Biggest Answers in Huntington's Disease. *International Journal of Molecular Sciences*, *19*(8), 2398. doi:10.3390/ijms19082398 URL: https://www.mdpi.com/1422-0067/19/8/2398

Salas-Vargas, J., Mancera-Gervacio, J., Velázquez-Pérez, L., Rodrígez-Labrada, R., Martínez-Cruz, E., Magaña, J. J., Durand-Rivera, A., Hernández-Hernández, O., Cisneros, B., & Gonzalez-Piña, R. (2015). Spinocerebellar Ataxia Type 7: A Neurodegenerative Disorder with Peripheral Neuropathy. *European Neurology*, *73*(3–4), 173–178. doi:10.1159/000370239 URL: https://www.karger.com/Article/Abstract/370239

Shao, J., & Diamond, M. I. (2007). Polyglutamine diseases: emerging concepts in pathogenesis and therapy. *Human Molecular Genetics*, *16*(R2), R115–R123. doi:10.1093/hmg/ddm213 URL:https://academic.oup.com/hmg/article/16/R2/R115/2357150

Stack, E. C., Kubilus, J. K., Smith, K., Cormier, K., del Signore, S. J., Guelin, E., Ryu, H., Hersch, S. M., & Ferrante, R. J. (2005). Chronology of behavioral symptoms and neuropathological sequela in R6/2 Huntington's disease transgenic mice. *The Journal of Comparative Neurology*, *490*(4), 354–370. doi:10.1002/cne.20680 URL: https://onlinelibrary.wiley.com/doi/10.1002/cne.20680

Sturtevant, A. H. (1959). Culture methods for *Drosophila*. *Culture Methods for Invertebrate Animals*, 437446

Srinivasan, S. R., de Gusmao, C. M., Korecka, J. A., & Khurana, V. (2023). Repeat expansion disorders. In Neurobiology of Brain Disorders (pp. 293-312). Academic Press.

Takahashi, T., Katada, S., & Onodera, O. (2010). Polyglutamine Diseases: Where does Toxicity Come from? What is Toxicity? Where are We Going? *Journal of Molecular Cell Biology*, *2*(4), 180–191. doi:10.1093/jmcb/mjq005 URL: https://academic.oup.com/jmcb/article/2/4/180/865830

Tsuji, S. (2012). Dentatorubral–pallidoluysian atrophy. *Handbook of Clinical Neurology*, 587–594. doi:10.1016/b978-0-444-51892-7.00041-3 URL: https://www.sciencedirect.com/science/article/abs/pii/B9780444518927000413?via%3Dihub

Tzou, F. Y., Wen, J. K., Yeh, J. Y., Huang, S. Y., Chen, G. C., & Chan, C. C. (2022). *Drosophila* as a model to study autophagy in neurodegenerative diseases and digestive tract. IUBMB life, 74(4), 339-360. doi: 10.1002/iub.2583 URL: https://iubmb.onlinelibrary.wiley.com/doi/10.1002/iub.2583.

Venken, K. J. T., & Bellen, H. J. (2005). Emerging technologies for gene manipulation in Drosophila melanogaster. Nature Reviews Genetics, 6(3), 167–178. doi:10.1038/nrg1553 URL:https://www.nature.com/articles/nrg1553

Warrick, J. M., Paulson, H. L., Gray-Board, G. L., Bui, Q. T., Fischbeck, K. H., Pittman, R. N., & Bonini, N. M. (1998). Expanded Polyglutamine Protein Forms Nuclear Inclusions and Causes Neural Degeneration in *Drosophila*. *Cell*, *93*(6), 939–949. doi:10.1016/s0092-8674(00)81200-3 URL: https://www.sciencedirect.com/science/article/pii/S0092867400812003?via%3Dihub

Watase, K. (2014). Spinocerebellar ataxia type 6: Lessons from faithful knock-in mouse models. *Neurology and Clinical Neuroscience*, *3*(1), 14–17. doi:10.1111/ncn3.137 URL: https://onlinelibrary.wiley.com/doi/10.1111/ncn3.137

Wouters, O. J., McKee, M., & Luyten, J. (2020). Estimated Research and Development Investment Needed to Bring a New Medicine to Market, 2009–2018. *JAMA*, *323*(9), 844. doi:10.1001/jama.2020.1166 URL: https://jamanetwork.com/journals/jama/fullarticle/2762311

Xu, Z., Joel Tito, A., Rui, Y. N., & Zhang, S. (2015). Studying polyglutamine diseases in *Drosophila*. *Experimental Neurology*, *274*, 25–41. doi:10.1016/j.expneurol.2015.08.002 URL: https://www.sciencedirect.com/science/article/abs/pii/S0014488615300637?via%3Dihub

Yamaguchi, M., & Yoshida, H. (2018). *Drosophila* as a Model Organism. *Advances in Experimental Medicine and Biology*, 1–10. doi:10.1007/978-981-13-0529-0_1 URL: https://link.springer.com/chapter/10.1007/978-981-13-0529-0_1

Yamamoto, S., Jaiswal, M., Charng, W. L., Gambin, T., Karaca, E., Mirzaa, G., Wiszniewski, W., Sandoval, H., Haelterman, N. A., Xiong, B., Zhanf, K., Bayat, V., David, G., Li, T., Chen, K., Gala, U., Harel, T., Pehlivan, D., Penney, S., Vissers, L. E. L. M., Ligt, J., Jhangiani, S. N., Xie, Y., Tsang, S. H., Parman., YSivaci, M., Battaloglu, E., Muzny, D., Wan, Y. W., Liu, Z., Lin-Moore, A. T., Clark, R. D., Curry, C. J., Link, N., Schulze, K. L., Boerwinkle, E., Dobyns, W. B., Allikmets, R., Gibbs, R. A., Chen, R., Lupski, J. R., Wangler, M. F., & Bellen, H. J. (2014). A *Drosophila* genetic resource of mutants to study mechanisms underlying human genetic diseases. *Cell*, *159*(1), 200-214. doi: 10.1016/j.cell.2014.09.002 URL: https://www.sciencedirect.com/science/article/pii/S0092867414011131

Yang, S., Chang, R., Yang, H., Zhao, T., Hong, Y., Kong, H. E., Sun, X., Qin, Z., Jin, P., Li, S., & Li, X. J. (2017). CRISPR/Cas9-mediated gene editing ameliorates neurotoxicity in mouse model of Huntington's disease. *Journal of Clinical Investigation*, *127*(7), 2719–2724. doi:10.1172/jci92087 URL: https://www.jci.org/articles/view/92087

Zhang, S., Binari, R., Zhou, R., & Perrimon, N. (2010). A Genomewide RNA Interference Screen for Modifiers of Aggregates Formation by Mutant Huntingtin in *Drosophila*. *Genetics*, *184*(4), 1165–1179. doi:10.1534/genetics.109.112516 URL: https://academic.oup.com/genetics/article/184/4/1165/6063056