

Review

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# Advantages and differences among various animal models of Huntington's disease

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## Abstract

Treating and preventing neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, dementia with Lewy bodies, amyotrophic lateral sclerosis, frontotemporal dementia, and Huntington's disease have become significant challenges in the field of neurological research. In the early stages of neurodegenerative disease research, scientists often choose appropriate animal models to delve deeper into their molecular pathological mechanisms and macroscopic clinical manifestations. Selecting the right animal model is a crucial step in initiating and advancing this research process. This article focuses on analyzing various animal models used in the field of neurodegenerative diseases in recent years, with a particular focus on Huntington's disease. It discusses in detail the advantages and disadvantages of different animal models in experimental research, as well as the pathological features and clinical manifestations they exhibit.

**Keywords:** Huntington's disease, neurodegenerative disease, *Caenorhabditis elegans*, *Drosophila melanogaster*, zebrafish, rodent, sheep, pig, animal models



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## INTRODUCTION

Animal models have played a pivotal role in the investigation of various neurodegenerative diseases, offering insights into disease pathogenesis, progression, and potential therapeutic targets. The selection of an appropriate animal model is crucial as different models possess distinct advantages and limitations. It is imperative to carefully consider the characteristics of each model, encompassing similarities in genetic mutations, phenotypic expression, and disease progression, to ensure that the chosen model accurately recapitulates the human disease and is suitable for the specific research objectives. This paper conducts an in-depth analysis of the merits and drawbacks of the diverse animal models extensively employed in Huntington's disease (HD) research over recent years, encompassing a comprehensive evaluation spanning from large to small animal models. Our endeavor is to equip novices entering the realm of HD research with a visionary perspective by meticulously elucidating the current landscape of animal model utilization in HD studies. Utilizing specialized biological terminology and adjusting phrasing for enhanced professionalism, we have constructed a systematic and comprehensive theoretical framework. This framework aims to serve as a robust theoretical foundation and guiding principles for future research endeavors, enabling them to accurately select the most apt animal model tailored to their specific research objectives.

## CHARACTERISTICS OF HD

HD is a dominant genetic disorder caused by mutations in the Huntington gene located on chromosome 4, clinically manifesting as choreiform movements accompanied by progressive cognitive and psychiatric dysfunction, ultimately leading to dementia, and in severe cases, even asphyxia and death. The pathogenic gene Huntingtin (*HTT*) in HD encodes the HTT protein, and its first exon contains repeated CAG triplets. In HD, the number of CAG triplet repeats abnormally increases, and individuals with more than 36 CAG repeat triplets will develop the disease. The N-terminal fragment of the *HTT* gene, containing more than 36 polyglutamine (poly Q) repeats in the CAG sequence, forms inclusions within the nucleus and aggregations in nerve fibers. A prominent hallmark of this condition is the formation of aggregates by the mutant HTT (mHTT) protein, which leads to striatal atrophy and significant loss of medium spiny neurons in patients. Currently, there is no effective treatment for HD, and clinical management is primarily focused on symptomatic relief. This ultimately leads to the patient's demise, occurring approximately 10 to 15 years after the initial signs of the disease manifest<sup>[1-3]</sup>.

The acknowledgment of polyQ's significance evolved gradually over time. By the late 1990s, researchers had already noted the instability of HTT CAG repeats in human brain tissue post-mortem, as well as in a substantial proportion of rodent models. As investigations deepened, it emerged that the phenotype was primarily dictated by the length of the CAG repeat, rather than the length of the polyQ tract within the Huntington protein<sup>[4]</sup>. This revelation also sheds light on why certain rodent models, notably YAC128 and early BACHD mice, failed to fully replicate the observed phenotype (owing to the presence of CAA interruptions within the CAG repeats in the original YAC and BAC constructs). These interruptions mitigated the somatic instability of CAG repeats, while both CAA and CAG code for the same amino acid, glutamic acid. A recent pre-print paper from Steve McCarroll's lab similarly supports the idea that somatic instability plays a key role in HD pathogenesis<sup>[5]</sup>. Therefore, paying attention to the instability characterization of animal models of different animals is an important aspect in selecting suitable animal models for research.

While scientists have made significant strides in understanding HD, finding effective treatments to halt or slow its progression remains a distant goal. The gradual nature of the disease necessitates extensive and costly clinical trials. Additionally, the limited availability of HD patients for such trials restricts the number

of biochemical components that can be tested at any given time. Consequently, animal models occupy a pivotal position in HD research. They serve as a vital gateway before embarking on human clinical trials and as a tool to identify potential therapeutic compounds with promising outcomes.

Animal models of HD have a long history, and existing animal models encompass rhesus monkey, crab-eating macaque, marmoset, sheep, miniature pig, dog, rat, mouse, song bird, frog, zebrafish, fruit fly, roundworm, amoeba, bacteria, budding yeast, *etc.*<sup>[6]</sup>. The classification of animal models includes higher mammals, rodents, birds, amphibians and fish, invertebrates, and unicellular microorganisms. These animal models provide researchers with diverse perspectives. It is impractical to provide a comprehensive overview of every animal model in a single article, so we have selected a variety of common large and small animal models for a brief overview, which should meet the needs of most researchers [Figure 1].

## EARLY ANIMAL MODELS

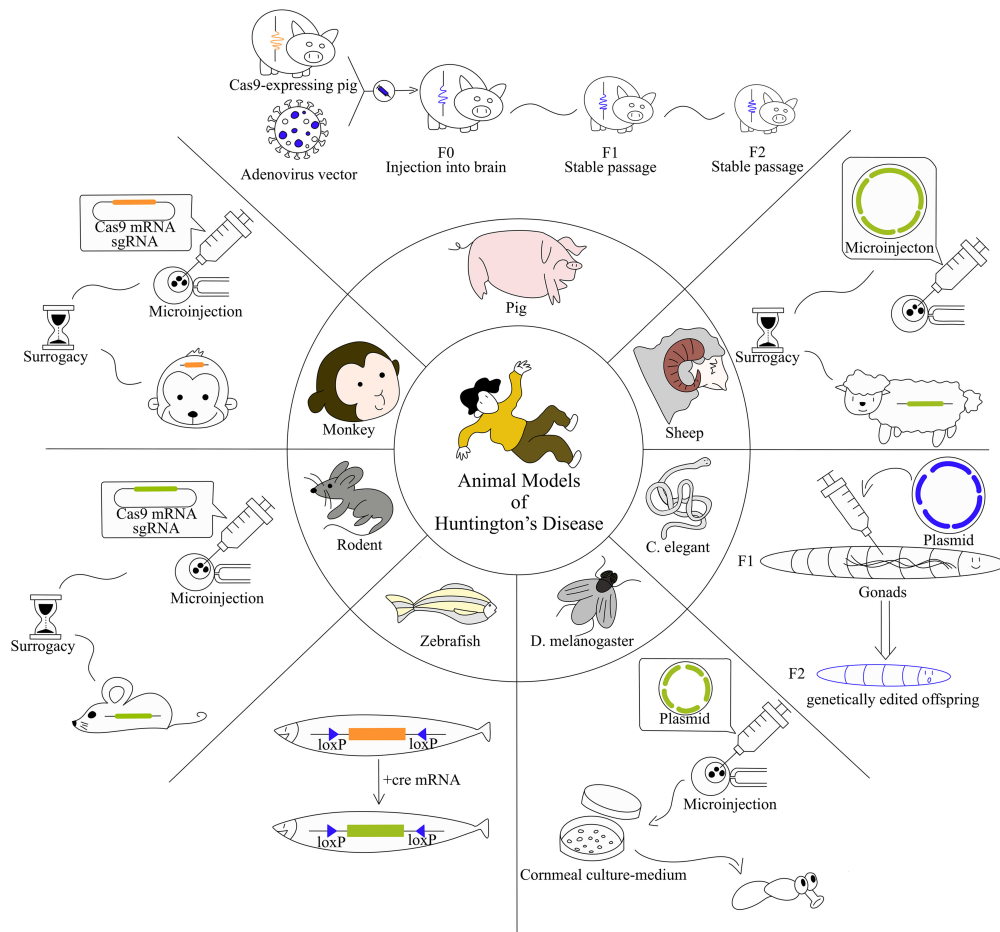
In the early stages of this disease research, the modeling of HD primarily relied on acute striatal atrophy induced by neurotoxic agents<sup>[7-9]</sup>. Although striatal atrophy is considered a key neuropathological feature of HD, mHTT is widely expressed throughout the central nervous system and can also be found in peripheral tissues<sup>[10,11]</sup>. Therefore, models centered solely on acute striatal damage are insufficient to comprehensively reveal the complex manifestations of the disease in these aspects. The limited pathological scope of striatal lesions makes it difficult to replicate many HD neurological symptoms caused by lesions in other brain regions in current disease models. In recognition of this, researchers have begun to shift their modeling focus toward the development of genetic animal models, aiming to delve deeper into the essence of the disease.

## CAENORHABDITIS ELEGANS MODELS

The *Caenorhabditis elegans* (*C. elegans*) nematode, a non-parasitic species that primarily feeds on bacteria, boasts a nervous system that has been comprehensively and meticulously described. Each neuron's position and connectivity within this system have been accurately mapped, providing a detailed understanding of its neurobiological structure<sup>[12]</sup>. Remarkably, *C. elegans* maintains its transparency throughout its entire lifecycle, enabling the utilization of fluorescent markers for precise tracking and analysis. Its small size further facilitates non-invasive optical monitoring and manipulation techniques, enabling researchers to delicately observe and manipulate its biological processes<sup>[13]</sup>. Moreover, the fully sequenced genome of *C. elegans*, measuring a mere 97 Mb, reveals that 60%-80% of its genes have homologs in humans, highlighting its significance as a model organism for studying diverse biological phenomena and diseases<sup>[14]</sup>.

In the experiment, the edited progeny of *C. elegans* can be obtained by injecting edited gene fragments into the gonads of *C. elegans* with plasmids as carriers<sup>[15]</sup>. In addition, a diverse group of scientists have made concerted efforts to unpack the molecular biology behind HD by expressing polyQ tracts with varying repeat lengths in specific neuronal subtypes of *C. elegans*. A pivotal aspect of their methodology involves integrating amino acids sourced from both standard and amplified human HTT with fluorescently tagged proteins in the touch receptor neurons of *C. elegans*. This enables them to corroborate the formation of protein aggregates and other pertinent clinical manifestations<sup>[16-18]</sup>.

Even though there is no HTT orthologue present in *C. elegans*, HTT can be expressed in *C. elegans* using a transgenic formula to induce age-related mechanosensory impairment, neuronal dysfunction, and neurodegeneration under sensitized conditions<sup>[19]</sup>. *C. elegans* has been widely used to model several aspects of polyQ cytotoxicity and to identify novel disease-modifying factors. Not only that, they are also used in classical genetic screening. In the study by Faber *et al.*, through genetic screening techniques, the *C. elegans*



**Figure 1.** Various animal models and modeling methods used in Huntington's disease research. Common animal models include both large and small animals, which are often created using viruses or plasmids as vectors through injection. The targets of the injection can range from fertilized eggs to the brain.

model was used to identify proteins that protect neurons from the toxic effects of extended polyQ tracts<sup>[20]</sup>. These models have also been extensively employed to delve into the underlying mechanisms of HD and rigorously evaluate the therapeutic promise of diverse compounds. Voisine *et al.* used an invertebrate model instead of a mammalian model to shorten the testing time from months to days, revealing the neuroprotective effects of lithium chloride and mithramycin in HD<sup>[21]</sup>. Other substances found to help prevent HD based on the model include rutin<sup>[22-24]</sup>, xyloketal B<sup>[25]</sup>, and diphenyl diselenide<sup>[26]</sup>.

Indeed, the *C. elegans* serves as an outstanding research platform at the molecular level. Nevertheless, the drawbacks of these models are quite evident. Chief among them is the inability to replicate identical clinical symptoms observed in humans, given that *C. elegans* lacks peripheral tissues resembling those of humans and possesses a central nervous system vastly different from humans. Consequently, it is infeasible to inform a patient that a potential therapy deemed effective in a nematode can be directly translated into human therapy.

## **DROSOPHILA MELANOGASTER MODELS**

The *Drosophila melanogaster* (*D. melanogaster*) has served as a genetic model system for scientific research for over a century. Due to its low maintenance and rapid reproduction costs, researchers have developed numerous genetic techniques over the years<sup>[27]</sup>. The high degree of genetic homology between *D. melanogaster* and humans makes it a valuable model for exploring cellular and molecular mechanisms underlying developmental processes and diseases. In recent years, *D. melanogaster* has successfully been used to mimic various human diseases, including numerous neurological disorders<sup>[28,29]</sup>. Its nervous system is complex and diverse, exhibiting many features similar to humans, such as eyes, olfactory, gustatory, and auditory organs, as well as a ventral nerve cord analogous to the spinal cord. Furthermore, it possesses peripheral sensory neurons for proprioception and pain perception, along with a brain<sup>[30-36]</sup>. Notably, a significant number of mutants affecting neural development have been generated in *D. melanogaster*, providing researchers with a rich resource for investigation<sup>[37,38]</sup>.

Despite the absence of an expanded polyQ sequence in the amino-terminal domain of *D. melanogaster*'s HTT protein, there exists a remarkable sequence conservation with the human protein, exhibiting approximately 49% positive homology and 27% identity<sup>[39]</sup>. This conserved sequence predicts a shared folding pattern between the two proteins. As a result, *Drosophila* serves as a valuable model organism for studying HD. By introducing mutated human *HTT* genes into *Drosophila*, researchers can explore the consequences of these mutations on protein function and interactions within the cell. When constructing *D. melanogaster* models for HD, most mutations are designed based on fragments of the HTT protein. Some studies choose to express only specific gene fragments, while others opt for larger fragments<sup>[40-42]</sup>.

A pivotal stage in the development of HD involves the cleavage of the full-length HTT protein. This process liberates N-terminal fragments of varying lengths, containing an elongated polyQ tract that ultimately becomes neurotoxic in neuronal cells, contributing significantly to the pathogenesis of the disease<sup>[43]</sup>. The production of both N-terminal and C-terminal fragments, the latter arising specifically from double proteolysis, significantly augments toxicity mechanisms in HD. These byproducts contribute to the dilation of the endoplasmic reticulum (ER) and intensify ER stress, thereby impairing the crucial activity of dynamin 1 protein and ultimately triggering cell death. It was experimentally demonstrated that the N-terminal and C-terminal metabolites produced by HTT protein hydrolysis in *Drosophila* model exhibited toxicity in the organism<sup>[39]</sup>.

Transgenic *D. melanogaster* has proven effective in simulating some of the core features of HD, including a significantly shortened lifespan, the late-onset nature of the illness, motor dysfunction, and the gradual accumulation of aggregates within neurons, axons, and the cytoplasm. Chongtham *et al.* investigated the role of changes in gut microbiota composition in the pathogenesis of several neurodegenerative disorders, with a particular focus on the progression of HD. Using transgenic *D. melanogaster* models expressing full-length or N-terminal mutant fragments of human HTT protein, the study aimed to explore how gut bacteria influence the progression of HD<sup>[44]</sup>. The *Drosophila* model has also been used to evaluate potential treatments in HD, with a 2018 study showing that *Rhodiola rosea* improved several measures in flies expressing HTTQ93<sup>[45]</sup>.

These models enable researchers to gain insights into the disease's pathogenesis and progression, thereby facilitating the development of potential therapeutic strategies. Moreover, this approach allows researchers to investigate the effects of different regions of the HTT protein and their contribution to the pathogenesis of HD. By studying these models, scientists can gain insights into the molecular mechanisms underlying the disease and potentially develop new therapeutic strategies.

## ZEBRAFISH MODELS

With its unique advantages of large embryonic size, short developmental time, and transparency, the zebrafish has emerged as a popular model organism in vertebrate developmental studies. The large size of its embryos facilitates ease of observation and manipulation, while the short developmental cycle allows for the rapid exhibition of various changes throughout development<sup>[46]</sup>. Furthermore, the transparency of the zebrafish embryo enables the direct observation of internal structures and cellular dynamics without the need for special treatments. These characteristics collectively contribute to the zebrafish's status as an ideal choice for studying the developmental mechanisms of vertebrates, providing scientists with valuable experimental materials and observation windows<sup>[47]</sup>.

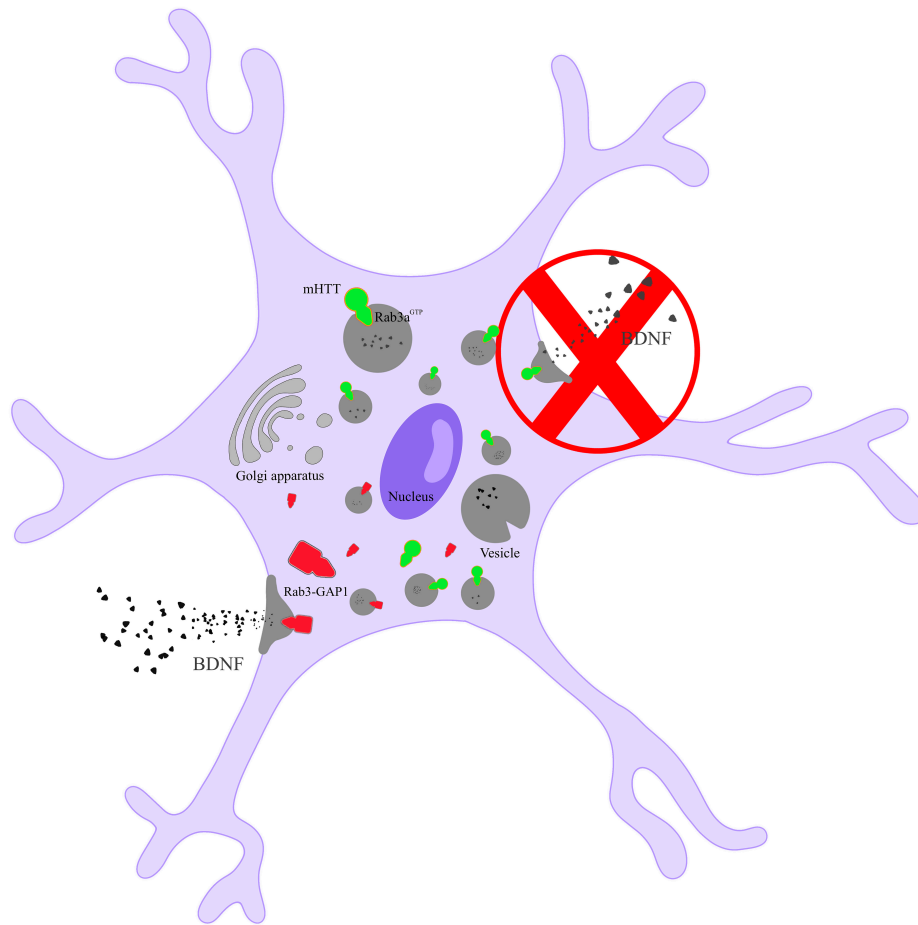
The HTT homolog in zebrafish is similar in size to human HTT and exhibits a high degree of identity, reaching up to 70% in amino acid sequence<sup>[48]</sup>. From a holistic perspective, the absence of HTT has a significant impact on the developmental process of zebrafish embryos<sup>[49]</sup>. This discovery further emphasizes the crucial role of HTT in vertebrate development and provides strong support for utilizing zebrafish as a model for studying neurodegenerative diseases such as HD. In the zebrafish system, expressing normal and expanded polyQ fragments of HTT through gene editing techniques has become a commonly adopted research method<sup>[50]</sup>. In these cell lines, the length of the polyQ fragment is closely associated with its misfolding, oligomerization, aggregation, and toxic manifestations, which are highly consistent with observations in other animal models and patients. Insufficient expression of HTT in zebrafish can lead to various developmental defects and disruption of iron homeostasis<sup>[49]</sup>. Furthermore, recent studies have demonstrated that phosphodiesterase 5 (PDE5) inhibitors upregulate intracellular cGMP levels, resulting in a reduction of aggregates with 71 glutamine repeats in zebrafish larvae expressing human HTT<sup>[51]</sup>.

When exploring the complex pathological mechanism of HD, a crucial molecule, brain-derived neurotrophic factor (BDNF), has entered the field of view of scientists, and several research teams have tried to unravel the mechanism of BDNF in HD through zebrafish models. BDNF, an integral member of the neurotrophin family, exhibits a notable diversity in its isoforms, with three distinct variants identified in zebrafish<sup>[52]</sup>. The initiation of BDNF signaling, a pivotal process in neuronal health, commences with BDNF transcription, which is disrupted in HD. This transcriptional disruption stems from a decreased BDNF level, a consequence of the CAG repeat expansion in the HTT. Specifically, the CAG repeat in HTT disrupts the normal interaction between BDNF and RE1-silencing transcription factor (REST). This disruption facilitates REST's nuclear translocation, leading to a suppression of BDNF expression<sup>[53]</sup>. This downregulation of BDNF, in turn, has detrimental effects on spiny projection neurons, a crucial neuronal population in HD. Furthermore, HTT interferes with the axonal transport of BDNF-containing vesicles. This interference, likely due to altered binding dynamics between dynactin, microtubules, and other components of the transport machinery<sup>[54]</sup>, further exacerbates the deficit in BDNF signaling within HD-affected neurons [Figure 2]. Thus, multiple mechanisms converge to impair BDNF signaling in HD, underlining its central role in disease progression and highlighting potential therapeutic targets.

Zebrafish model has been playing an important role in the field of HD research, and scientists expect to further study the pathogenesis and development model of HD through this model. However, due to the lack of some important human tissues and organs, resulting in a very different drug delivery route from humans, researchers usually choose rodent models when studying the clinical manifestations and pathological characteristics of HD.

## RODENT MODELS

For a long time, and even now, many scientists have created rodent models of HD by injecting acute striatal



**Figure 2.** mHTT competitively inhibits the release of BDNF, which causes nerve damage. mHTT: Mutant Huntingtin; BDNF: brain-derived neurotrophic factor.

damage caused by chemical toxins. Intraventricular injection of quinolinic acid, an NMDA receptor agonist, can replicate the striatal histopathology of HD, making it one of the most popular methods for HD modeling. However, the nature of the pathogenesis of this modeling method is not the same; perhaps we can observe similar clinical manifestations with HD, but can not deeply study the pathogenesis of HD at the molecular level.

With the completion of the Human Genome Project and the sequencing of the *Mus musculus* genome, researchers have discovered significant genetic homology between these species. Concurrently, the rapid advancements in biotechnology and the successful breeding of numerous knockout and transgenic mouse strains have established mice as the most widely used model organisms for studying human diseases. Furthermore, from practical and economic perspectives, mice are favored by scientists due to their small size, rapid reproduction rate, and ease of manipulation, making them important experimental subjects. Currently, the rodent models used in HD research are mainly divided into Truncated N-terminal fragment models and Full-length HD models. The latter is divided into knock-in models and transgenic models [Table 1].

**Table 1. Application of a partial rodent model to HD**

Model	Transgene product	Phenotype		Survival	Ref.
		Neuropathology	Survival and general observations		
<b>Truncated N-terminal fragment models</b>					
N171-82Q	171 amino acids of N-terminal fragment	Internal inclusion bodies and neuritis aggregates		20-24 weeks	[55]
R6/1	67 amino acids of N-terminal fragment (116 CAG)	Progressive brain atrophy Decline in the number of neurons		32-40 weeks	[56,57]
R6/2	67 amino acids of N-terminal fragment (144 CAG)	Decreased striatal and brain size		12-15 weeks	[57,58]
HD51	N-terminal fragment (22% of rat <i>HTT</i> gene)	Significant nuclear aggregates and inclusion bodies		98 weeks	[59-61]
N118-82Q	118 amino acids of N-terminal fragment	\		28-40 weeks	[62,63]
N586-82Q	586 amino acids of N-terminal fragment	\		32-36 weeks	[64,65]
<b>Full-length HD models: transgenic models</b>					
HD89	Full-length human <i>HTT</i>	Neuronal loss and astrocytoma		Normal lifespan	[66]
YAC128	Full-length human <i>HTT</i>	Reduced striatum and cortex volume, Neuron loss and dysfunction		Normal lifespan	[67-71]
BACHD	Full-length human <i>HTT</i>	Delayed atrophy of the lamina and striatum		Normal lifespan	[60]
<b>Full-length HD models: knock-in models</b>					
Hdh4/Q72, Hdh4/Q80	Full-length chimeric human <i>HTT</i> exon 1:mouse <i>HTT</i>	Increased levels of benzodiazepine receptor binding sites in the striatum and cerebral cortex		Normal lifespan	[72,73]
HdhQ111	Full-length chimeric human <i>HTT</i> exon 1:mouse <i>HTT</i>	Changes in striatal neurons		Normal lifespan	[74-76]
HdhQ140	Full-length chimeric human <i>HTT</i> exon 1: mouse <i>HTT</i>	Striatal <i>HTT</i> nuclear staining. Nuclear and neuropil aggregates prominent in the striatum, nucleus accumbens, and olfactory tubercle, as well as widespread nuclear staining including cortical layers II/III, and V., hippocampus, and regions of the olfactory system		Normal lifespan	[74]
HdhQ150	Full-length chimeric human <i>HTT</i> exon 1:	Striatal nuclear <i>HTT</i> immunoreactivity. Striatal NIs. Reactive astrocytic gliosis. Robust striatal NIs and striatal neuron receptor loss. Loss of striatal perikarya		Normal lifespan	[77-79]



mouse HTT

and volume. Decrease in striatal dopamine D1 and D2 receptors and dopamine transporters

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HD: Huntington's disease; HTT: Huntingtin.

Compared to toxin-induced models, genetic models offer a more specific and in-depth understanding of the behavioral and physiological progression of HD phenotypes<sup>[80]</sup>. Among them, the R6/2 transgenic mouse model stands out as one of the most commonly used, characterized by its aggressive and rapidly progressing phenotype with a short lifespan of approximately 15 weeks<sup>[57]</sup>. These mice begin to exhibit symptoms such as irregular gait, claspings of hind limbs, weight loss, increased grooming behavior, and cognitive decline at around 5 weeks of age. Furthermore, as transgenic mice age, they become increasingly prone to seizures, further mimicking the complex pathological processes of HD. The N171-82Q model incorporates specific fragments of the *HD* gene's N-terminal region, including exons 1 and 2, expressing the first 171 amino acids with 82 glutamine repeats<sup>[55]</sup>. While this model also displays pathological changes such as striatal atrophy, ventricular enlargement, and failure to gain weight in later stages of the disease, it is notable for not exhibiting hyperkinesia or epileptic activity<sup>[81,82]</sup>. Additionally, these mice have a relatively longer lifespan of approximately 18-25 weeks.

The above two rodent models are N-terminal models. One of the limitations of utilizing N-terminal models is their inability to be studied over an extended period due to their relatively short lifespan. Additionally, they do not encompass the full-length gene, thereby lacking crucial regulatory elements that may contribute to a comprehensive understanding of the disease. Full-length models, such as YAC128 or BACHD, offer new perspectives on disease research by introducing complete human mHTT transgene. The YAC128 mouse model is particularly unique in that it carries 128 CAG repeats from the human *HTT* gene<sup>[67]</sup>, which more accurately simulates the disease state. These mice usually begin to show abnormal behavior at about 6 months of age, and by about 12 months, the striatal neurons have selectively degraded, a process that reflects the gradual progression of the disease. Specifically, the disease progressed as the mice experienced brief weight gain at 2 months of age, followed by increased activity at 6 to 12 months, difficulty walking on a rotating rod, and eventually decreased movement. Notably, the YAC128 model showed significant loss of striatal medium-sized spiny neurons at 12 months and was accompanied by cognitive dysfunction and increased mHTT aggregation at 18 months. The advantage of full-length models is that they support long-term research into the disease, which is essential to explore long-term treatment strategies. Due to the inclusion of complete human genes, these models also include all relevant regulatory elements, making the findings more closely related to the real situation of human disease.

Knock-in models differ from transgenic models mainly in that they can express specific mutations in endogenous genomic locus rather than through the introduction of foreign genes. Multiple KI models based on CAG repeat length have been successfully created by replacing alleles in the first exon of the mouse *HTT* gene with human mutated variants. Because KI models are able to more accurately simulate the genetic background of human HD, they exhibit a high degree of phenotypic similarity to human HD. In addition, KI models typically have a longer lifespan and are capable of progressively exhibiting disease-related symptoms, which makes them extremely valuable in HD research, especially as they provide an ideal platform for evaluating the potential of long-term stem cell transplantation therapy.

In rodent models of different genotypes, scientists made a number of key discoveries. Geraldine Kong *et al.* confirmed the presence of gut dysbiosis in HD by observing the intestinal microbiome composition characteristics of the R6/1 transgenic mouse model of HD<sup>[83]</sup>. By studying the symptoms of R6/2 and Q175 HD mouse models, Tong *et al.* revealed that astrocytes and Kir4.1 channels were therapeutic targets<sup>[84]</sup>. Abjean *et al.* studied the symptoms of Hdh140 mice, confirming that the JAK2-STAT3 pathway controls the beneficial protein-homeostasis response of reactive astrocytes in HD<sup>[85]</sup>. HD animal models have also played a huge role in the field of therapeutic environments and drug interventions. Ona *et al.* found that inhibiting caspase-1 slowed disease progression in a mouse model of Huntington's disease<sup>[86]</sup>. van Dellen *et al.* used HD R6/1 mice to demonstrate that exposure to a stimulation-rich environment from an early age helps prevent brain volume loss and delays the onset of dyskinesia<sup>[87]</sup>. Ferrante *et al.* proposed that creatine has a neuroprotective effect on transgenic mouse models of HD<sup>[88]</sup>. The utilization of the YAC128 model has demonstrated the persistence of mHTT inclusions even after the manifestation of behavioral and neuropathological alterations<sup>[67]</sup>.

Transgenic full-length models, transgenic fragments, and knock-in models have all demonstrated significant effectiveness in their construction, effectively expressing the expanded polyQ repeat domain, which is a core factor in neurodegeneration in HD and other polyQ diseases. Additionally, the high similarity between the huntingtin protein and its homolog in mice suggests that they likely share similar functional properties. To increase the likelihood of predicting human outcomes from model results, the selection of appropriate rodent genetic HD models can be evaluated using the three criteria proposed by Paul Willner<sup>[89]</sup>. When planning an investigation, in addition to clarifying the research objectives, it is crucial to carefully consider the duration of the study, the achievable sample size, and the degree of relevance between the content studied and human HD in biochemical and behavioral aspects. Furthermore, the issues of consistency and stability arising from genetic variations introduced by breeding and background strains are also important considerations that cannot be overlooked.

## SHEEP MODELS

Despite the numerous advantages of rodent models, their brains do not exhibit apoptosis or significant neurodegenerative changes, which are crucial pathological features in human diseases. This is one of the key issues that cannot be avoided when discussing whether to choose rodent models as the subject of research. Therefore, scientists have gradually shifted their focus to larger animal models.

Jacobsen *et al.* developed a transgenic sheep model of HD to demonstrate the preclinical stage of the disease, aid in the study of early molecular and neuropathological changes occurring in Huntington's chorea, and test potential therapeutic approaches. The HD sheep were successfully developed by inserting the full-length human HTT cDNA containing 73 CAG repeats into a pronucleus, and then implanting this pronucleus into surrogate sheep<sup>[90]</sup>. The transgenic sheep G0/5 strain exhibited significant expression of mutant HTT mRNA and protein throughout their entire brain. Clinically, this model primarily manifests as early-onset abnormalities in circadian rhythm, despite the absence of significant pathological changes in the brain<sup>[91,92]</sup>.

However, the transgenic sheep model exhibits only mild neuropathological changes and lacks significant clinical phenotypic features, making it difficult to delve deeper into the impact of observed HTT reduction on disease progression. Additionally, due to the long gestation period (approximately 4.7 to 5 months) and lifespan of sheep, as well as their seasonal breeding habits, which limit the number of offspring produced each year to 1 to 3, the time required to develop and study sheep populations and collect relevant data is significantly longer compared to smaller experimental animals. This not only increases the pressure on research funding and publication, but also results in a longer time frame for making significant contributions to the field, posing significant challenges to research progress and output.

## NON-HUMAN PRIMATE (NHP) MODELS

By utilizing lentiviral vector technology, the team successfully introduced a transgenic fragment of human HTT exon 1 containing 84 CAG repeats into monkeys, establishing the first transgenic monkey model of HD. All five live-born neonates carried the transgenic mutant *HTT* and *GFP* genes. Their pathogenesis closely resembled that of humans: monkeys carrying a CAG repeat sequence below the threshold exhibited normal health, while those with a higher number of CAG repeats developed symptoms that positively correlated with the degree of repeat expansion. The main clinical manifestations included motor dysfunction and premature death<sup>[93]</sup>.

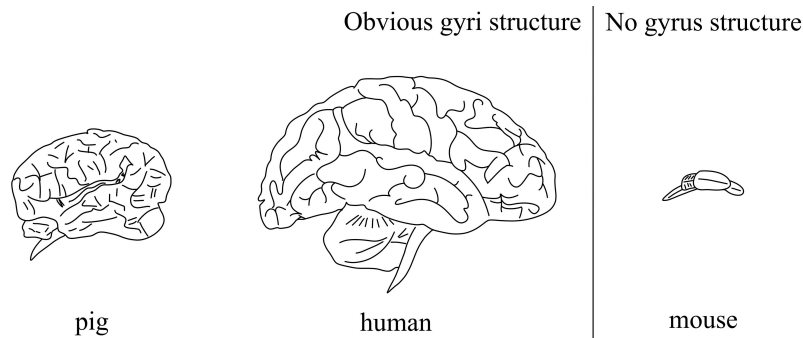
Subsequent in-depth research revealed that this monkey model not only spontaneously exhibited pathological manifestations such as motor dysfunction and epileptic seizures, but also gradually developed progressive cognitive decline. These features closely resemble the chronic course of adult HD, and the model exhibited particularly prominent HTT aggregates and axonal degeneration<sup>[94,95]</sup>. Compared to rodent models, the monkey model exhibited more severe phenotypic characteristics and a significantly shorter survival time. This phenomenon may stem from the higher sensitivity of primate neurons to toxic HTT protein fragments<sup>[96]</sup>. Therefore, this transgenic monkey model not only provides us with a research platform that is closer to the pathological process of human HD, but also offers valuable insights for our understanding of the pathogenesis of HD and the exploration of potential treatment strategies.

Similar to the sheep model, the monkey model also has a long gestation period, and even worse, they typically only have one offspring per pregnancy<sup>[97]</sup>. Coupled with the incomplete development of transgenic technology in monkeys, the creation of a monkey model is inefficient. These factors significantly increase the economic cost and research cycle of the study. Unlike other experimental animals, ethical considerations for non-human primates are much stricter, which greatly limits our ability to conduct more in-depth research on HD using monkey models<sup>[98]</sup>.

## PIG MODELS

In order to solve the problems in the above animal models, pig models have gradually entered the field of view of researchers. The neural anatomical structure of pigs is similar to that of humans. For example, they have a gyrus structure similar to that of humans. The dorsal striatum of pig brain is divided into two different structures by the internal capsule, just like that of humans. The sensory cortex of pigs, such as the motor cortex and somatosensory cortex, is arranged according to the position of the body, which is not found in rodent models [Figure 3]. Pigs also have many genetic and reproductive advantages that do not exist in primates, including a relatively short gestation period, multiple births, and a short time to sexual maturity. In addition, as an important part of the world's livestock industry, pigs are relatively easy to raise and the cost is relatively low.

In 2010, Yang *et al.* collaborated to establish the first transgenic HD pig model with the N-208-105Q mutation. This groundbreaking work revealed for the first time that the same mutant protein can induce cell apoptosis in the brains of pigs, a phenomenon that had not been observed in mouse brains previously. Furthermore, the model exhibited notable symptoms such as convulsions and choreiform movements. Unfortunately, transgenic pigs previously obtained through specific methods faced the challenge of extremely short survival times due to the severity of their clinical symptoms, greatly limiting further in-depth research<sup>[99]</sup>. Given this, the aforementioned research team once again collaborated to successfully construct an *HD-KI* gene knock-in pig model in an effort to overcome this challenge. This HD-KI pig



**Figure 3.** The gyrus of pigs is similar to that of humans, while that of rodents is not.

model exhibited specific neuronal death in the striatum, highly similar to the pathological changes observed in HD patients. Additionally, researchers observed a clear process of cell apoptosis. Notably, the HD-KI pigs also exhibited clinical symptoms such as abnormal gait and respiratory difficulties, which are consistent with the manifestations seen in human HD patients<sup>[100]</sup>. Crucially, the gene knock-in pig model possesses the ability to stably transmit genetic and phenotypic traits to its offspring, maximizing the reproductive and genetic advantages of the pig model. This provides us with a consistent and stable research platform to further explore the pathogenesis of HD and potential treatment strategies. The HD-KI pig, with its remarkably similar clinical manifestations to humans and its low-cost feeding model, is poised to become one of the essential platforms for translating experimental research into clinical applications in the treatment of HD.

In addition, among numerous HD animal models, especially in pigs, longer CAG repeat lengths may be required to maintain severe disease phenotypes, which are less common in human cases. Despite the fact that genomic editing techniques, particularly CRISPR-Cas9, have revolutionized the field of animal modeling, including HD research, they also exhibit certain limitations. One notable concern is the potential for off-target effects, leading to unintended genomic alterations. Furthermore, CRISPR/Cas9 may also induce mosaicism, which is particularly significant in large animal models and studies that involve founder animals. These limitations have the potential to confound the interpretation of phenotypes observed in generated animal models.

### PIG MODELS ARE BECOMING INCREASINGLY IMPORTANT

Different animals provide unique advantages as research platforms for HD, but their limitations cannot be ignored. While models such as *C. elegans*, *D. melanogaster*, and zebrafish allow us to easily observe molecular-level manifestations and pathological features similar to humans, such as premature death and progressive neurodegeneration, researchers are unable to observe any clinical symptoms that appear in humans, such as choreiform movements [Table 2]. Observing clinical manifestations in rodent and large animal models is generally a better choice because they possess highly homologous genes to humans and have more complex nervous systems. However, it is important to note that the choice of animal as the research subject, as well as the decision to use gene knockout or transgenic methods to create the model, can significantly affect the clinical symptoms observed.

In HD, we have to stress the importance of pig models in current research progress. As a model with the advantages of low-cost feeding, high litter size, and short gestation period, its advantages are particularly prominent in the brain gyrus structure similar to that of humans, and it has the same progressive characteristics, premature death, and various behavioral disorders as humans. In the widely used HD-KI

**Table 2. Comparison of various animal models in HD**

Species	Molding method	Pathological and clinical symptoms	Disadvantage	Ref.
<i>C. elegans</i>	Expressing fluorescently labeled polyQ proteins in neurons or muscle cells	Motility defects or ASH neurodegeneration	No pathologic processes and clinical symptoms; No orthogonal HTT	[17,101]
<i>D. melanogaster</i>	Using the GAL4/UAS (upstream activating sequence) system	Cytopathia caused by polyQ; The course of the disease is delayed and progressive; Loss of motor function, metabolic imbalance and premature death	A winged arthropod; differences in morphological development and movement patterns	[102-109]
Zebrafish	Genetic model (HTT)	Morphological abnormalities and necrosis of the central nervous system	Lack of certain vital human tissues and organs; Controlled route of administration	[110,111]

HD: Huntington's disease; *C. elegans*: *Caenorhabditis elegans*; ASH: amphid neurons single; HTT: Huntingtin; *D. melanogaster*: *Drosophila melanogaster*.

model, we can achieve efficient gene editing by injecting adenoviral vectors carrying gene fragments into the brains of Cas9-expressing pigs. This method perfectly reproduces the genetic manifestations of autosomal dominant inherited diseases. Because their offspring have a high survival rate and can be stably transmitted, researchers can more easily formulate reliable long-term experimental plans for behavioral analysis and the like.

## PERSPECTIVES

Regardless of the animal model chosen for research, the ultimate goal is always to serve clinical practice, aiming to provide beneficial insights and methods for the treatment of human diseases. Particularly in the study of HD, monkey and pig models have become increasingly popular and widely adopted due to their significant physiological similarities to humans. Utilizing these stable and reliable animal models to delve deeper into the pathophysiological mechanisms of HD from a developmental perspective holds immense research promise, undoubtedly ushering in a new era for HD research.

Furthermore, the rapid advancements in gene therapy and editing techniques have increasingly unlocked their potential to correct genetic defects during embryogenesis. As gene therapy gains wider acceptance, these technological advancements have sparked hope for correcting inherited diseases before birth. Therefore, by exploring the role of early HD neurodevelopmental disorders in the progression of the disease, we can not only pave new research directions for a more comprehensive understanding of HD, but also establish a solid foundation for exploring novel therapeutic strategies for HD.

## DECLARATIONS

### Authors' contributions

Wrote the review paper: Yu Z

Revised manuscript: Yang W, Yan S

Conceived and designed experiments: Yan S, Yu Z

All authors read and approved the final manuscript.

### Availability of data and materials

Not applicable.

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### Conflicts of interest

Yan S is a guest editor of the journal *Ageing and Neurodegenerative Diseases*, while the other authors have declared that they have no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

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