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Large animal models for Huntington's disease research

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ABSTRACT

Huntington's disease (HD) is a hereditary neurodegenerative disorder for which there is currently no effective treatment available. Consequently, the development of appropriate disease models is critical to thoroughly investigate disease progression. The genetic basis of HD involves the abnormal expansion of CAG repeats in the huntingtin (*HTT*) gene, leading to the expansion of a polyglutamine repeat in the HTT protein. Mutant HTT carrying the expanded polyglutamine repeat undergoes misfolding and forms aggregates in the brain, which precipitate selective neuronal loss in specific brain regions. Animal models play an important role in elucidating the pathogenesis of neurodegenerative disorders such as HD and in identifying potential therapeutic targets. Due to the marked species differences between rodents and larger animals, substantial efforts have been directed toward establishing large animal models for HD research. These models are pivotal for advancing the discovery of novel therapeutic targets, enhancing effective drug delivery methods, and improving treatment outcomes. We have explored the advantages of utilizing large animal models, particularly pigs, in previous reviews. Since then, however, significant progress has been made in developing more sophisticated animal models that faithfully replicate the typical pathology of HD. In the current review, we provide a comprehensive overview of large animal models of HD, incorporating recent findings regarding the establishment of HD knock-in (KI) pigs and their genetic therapy. We also explore the utilization of large animal models in HD research, with a focus on sheep, non-human primates (NHPs), and pigs. Our objective is to provide valuable insights into the application of these large animal models for the investigation and treatment of neurodegenerative disorders.

Keywords: Huntington's disease; Large animal models; Sheep; Non-human primates; Transgenic pigs

INTRODUCTION

Huntington's disease (HD) is a fatal, autosomal dominant inherited neurodegenerative disease that typically presents with motor impairments, including chorea, and cognitive decline (Bates et al., 2015; Ross & Tabrizi, 2011). Epidemiological reports reveal that the disease affects 2.7 out of every 100 000 people worldwide, with its prevalence varying significantly across different regions of the world. The incidence rate is lowest in Asian populations and highest in Caucasian populations (Medina et al., 2022; Rawlins et al., 2016). The average age of onset for HD is 40 years old. After onset, patients experience irreversible clinical deterioration, with an average survival period ranging from 15 to 20 years (Ghosh & Tabrizi, 2018). Juvenile HD patients are relatively uncommon, comprising only about 5% of HD patients (Cronin et al., 2019). Typically, they begin to develop the disease before the age of 21 and experience rapid deterioration, with death occurring approximately 10 years after onset (Quigley, 2017; Saudou & Humbert, 2016). Consequently, individuals with HD who develop symptoms during adolescence experience more rapid disease progression compared to those who develop symptoms in adulthood (Foroud et al., 1999).

HD is characterized by neuronal loss and brain atrophy, particularly in the striatum of the basal ganglia (Parsons & Raymond, 2015). As the disease advances, patient brains exhibit varying degrees of atrophy in the cerebral cortex, basal ganglia structures, thalamus, white matter, and cerebellum (Kassubek et al., 2004), with brain weight decreasing by up to 25% in later stages of the disease (Roos, 2010). Huntington's disease not only affects the central nervous system, but also peripheral tissues such as the cardiovascular system, lungs,

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muscles, and gastrointestinal tract, resulting in a range of symptoms such as heart failure, muscle wasting, osteoporosis, and circadian rhythm disruption (Her et al., 2015; Kwan et al., 2012; Mielcarek, 2015; Reyes et al., 2014; Roos, 2010; Wasser et al., 2020). While there are no existing treatments to modify the course of the disease, extensive research in the field of polyglutamine (polyQ) diseases has uncovered a broad range of deleterious effects of expanded polyQ on diverse cellular mechanisms and functions (Bradford et al., 2010; Friedman et al., 2007). This understanding has led to the prevailing theory that inhibiting the expression of expanded polyQ proteins may be an effective approach to curing these diseases (Yan et al., 2023).

Nevertheless, our understanding of the pathogenic manifestations remains incomplete, partly due to the ubiquitous expression of HTT in various tissue types and its intricate role in diverse molecular pathways. Recent genome-wide association studies (GWAS) have identified several crucial genes involved in DNA repair that, along with genetic modifiers, add complexity to the age of onset and contribute to the somatic instability of CAG pathogenic repeats (Jiang et al., 2023; Thompson & Orr, 2023). Medium spiny neurons (MSNs), GABAergic neurons located in the striatum of the brain, are among the most vulnerable to damage in HD. The currently proposed mechanism outlines damage to MSNs, potentially caused by a combination of glutamate excitotoxicity, mitochondrial dysfunction, and neuroinflammation (Victor et al., 2018). However, the specific reasons for the vulnerability of MSNs in HD are not yet fully understood. Controversy persists regarding whether the mechanism underlying gain-of-function is due to aberrant mutant HTT (mHTT) or dominant loss of wild-type HTT function; however, it is probable that a combination of both factors contributes to HD pathogenesis (Laundos et al., 2023a, 2023b; Schulte & Littleton, 2011; Tabrizi et al., 2019a). Moreover, there is a lack of effective interventions for HD, and the translation of research findings from HD models to human clinical trials has not been successful (Kim et al., 2021). These disappointing outcomes can be partly attributed to the heterogeneity of HD phenotypes, which include variations in symptoms and disease stages among individuals. Additionally, the absence of reliable clinical endpoints in these studies impairs the predictability and potential effectiveness of therapeutic approaches. Therefore, it is essential to develop more refined animal models that accurately capture the complex features of HD and to establish reliable biomarkers to evaluate disease progression and treatment efficacy.

Several organisms, including flies, worms, yeast, and more recently, zebrafish, have all been genetically engineered to express versions of the Huntingtin (HTT) protein that contain expanded polyQ repeats (Chongtham et al., 2018). These HD models display a range of phenotypes, such as premature death, neurodegeneration, and impairment of motor function, and are readily reproducible, making them suitable for high-throughput research and certain drug screening applications. However, due to the significant biological disparities between these species and humans, small animal models face challenges in accurately replicating typical pathological changes observed in HD patients, such as selective neuronal degeneration. Despite these limitations, animal models play a crucial role in investigating HD and developing therapeutic strategies, with small animal models yielding valuable insights into HD pathogenesis.

The development of CRISPR/Cas9 technology has facilitated the generation of large animal models of HD. CRISPR/Cas9 allows for the precise and efficient editing of DNA, and has been widely adopted due to its simplicity, efficiency, and precision (Cho et al., 2013; Mali et al., 2013). This technology has been used to study the function of genes, create animal models of human diseases, and develop potential therapeutic applications. Thus, CRISPR/Cas9 has the potential to revolutionize the fields of genetic engineering and disease treatment and continue to play an important role in research and medicine in the future (Ran et al., 2013).

PATHOGENESIS OF HD

Approximately thirty years have passed since the identification of the *HTT* gene responsible for HD (MacDonald et al., 1993). This gene, located on the short arm of chromosome 4, encodes a large α-helical protein. The N-terminal domain of the HTT protein contains a polyQ stretch encoded by a CAG repeat and multiple HEAT domains (Guo et al., 2018). Under a normal genotype, CAG trinucleotide repeats within the HTT gene occur between 10 and 35 times. However, in individuals with HD, the number of CAG repeats significantly increases, often exceeding 36 (Figure 1). Extensively distributed in the body, HTT plays a crucial role in embryonic development and is involved in multiple cellular activities, including vesicle transport, endocytosis, autophagy, and transcriptional regulation. Although the biology of HTT is not yet fully understood, the discovery of numerous proteins that interact with HTT suggests that it plays a central role in mediating multiple protein-protein interactions (Guo et al., 2018; Shirasaki et al., 2012). HTT is essential for early animal development (Nasir et al., 1995), and a reduction in HTT levels in young and adult mice results in changes in brain homeostasis, progressive behavioral deficits, bilateral thalamic calcification, and even acute pancreatitis (Dietrich et al., 2017;

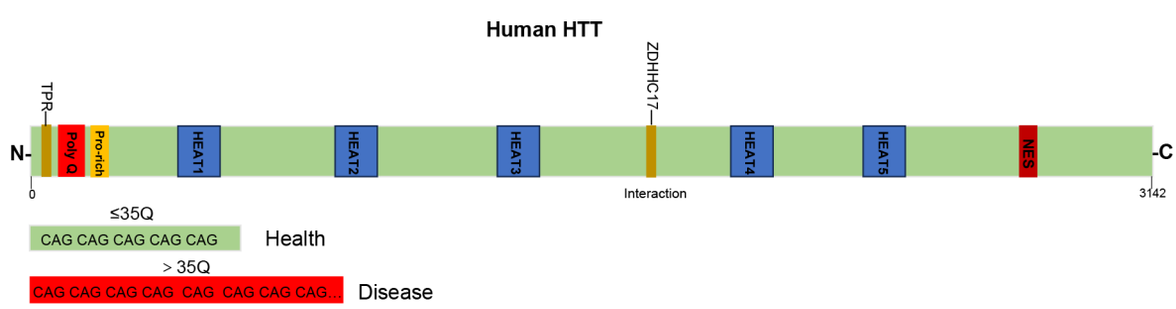

Figure 1 Schematic representation of huntingtin (HTT) protein structure

Huntingtin has a polyglutamine region (PolyQ) (red) and HEAT domains (blue). Large repeat numbers cause HD. N and C indicate N- and C-terminus of HTT; numbers indicate amino acid length of HTT.

Wang et al., 2016).

The N-terminal fragment encoded by exon 1, rather than full-length *HTT*, is considered the most toxic product involved in the pathophysiology of HD (Barbaro et al., 2015; Sathasivam et al., 2013). Notably, mHTT aggregates in the nucleus and cytoplasm of cells, forming inclusion bodies (IBs) (Landles et al., 2010). In mice, overexpression of N-terminal mHTT fragments leads to the accumulation of IBs in the striatum and the development of an age-dependent HD-like phenotype (Yang et al., 2020). This N-terminal fragment can be generated by aberrant mRNA splicing or proteolytic cleavage induced by polyQ expansion (Jarosińska & Rüdiger, 2021). The formation of mHTT aggregates depends on factors such as polyQ length, protein concentration, and time (Boatz et al., 2020). These aggregates present as different structures, such as monomers, oligomers, and IBs, which may affect proteins involved in energy metabolism, protein transport, RNA translation, and cell death (Ramdzan et al., 2017). These effects can lead to cellular dysfunction and slowed metabolism, eventually resulting in cell death. Although HTT is expressed widely throughout the body, the cytotoxic effects of mHTT are most pronounced in neural cells, particularly those in the striatum. This may be due to differences in the transcription levels of *HTT* in neural and non-neural cells (Didiot et al., 2018). Evidence suggests that neural cells are more prone to instability in CAG repeat sequences, resulting in excessive expansion of the CAG sequence. Additionally, compared to glial cells, neuronal cells are more vulnerable to HD-specific cytotoxicity, possibly due to differences in *mHTT* mRNA and protein levels in neuronal and glial cells (Kennedy & Shelbourne, 2000; Shelbourne et al., 2007).

ANIMAL MODELS FOR HD RESEARCH

Animal models play a pivotal role in elucidating disease pathology and physiology, as well as in the *in vivo* assessment of novel therapeutic drugs and treatments. These models are instrumental in advancing our understanding of disease mechanisms, evaluating the effectiveness of new therapies, and determining the most suitable treatment strategies. Animal models can be tailored to specific experimental requirements by selecting animals of different species, sex, and age, and by manipulating their genes to create models that closely resemble human diseases, enabling comprehensive study of the incidence, progression, and treatment of targeted diseases (McGonigle & Ruggeri, 2014).

As a monogenic mutation disease caused by CAG repeat expansions in the *HTT* gene, a variety of animal models carrying the *mHTT* gene have been established to mimic the HD phenotype (Crook & Housman, 2014; Ramaswamy et al., 2007), as presented in Table 1. Several comprehensive reviews have detailed the mouse models commonly used in HD research, which have provided valuable insights into the pathogenesis and treatment of HD (Ferrante et al., 2000; Lione et al., 1999; Mangiarini et al., 1996; Saydoff et al., 2006; Slow et al., 2005; Wang et al., 2008; Woodman et al., 2007). Studies using animal models have shown that small N-terminal HTT fragments carrying polyQ amplification are highly susceptible to misfolding and aggregation and exhibit greater toxicity than full-length mutant Htt (Schilling et al., 1999; Crook & Housman, 2011). Transgenic Htt mouse models expressing these small N-terminal Htt fragments exhibit earlier mortality and more severe behavioral phenotypes compared to those expressing full-length mutant HTT (Uchida et al., 2001; Baxa et al., 2013). However, despite showing age-dependent accumulation of mutant HTT and associated neurological symptoms, these HD mouse models do not typically present with clear, selective neurodegeneration, a pathological hallmark of HD (Crook & Housman, 2011; Levine et al., 2004). This phenomenon is not unique to HD mouse models; other genetically modified mouse models expressing various types of misfolded proteins, such as those for Alzheimer's disease (AD) and Parkinson's disease (PD), also fail to show clear, selective neurodegeneration, despite the presence of associated neurological symptoms (Tu et al., 2015; Yang et al., 2021). These discrepancies likely arise from significant interspecies differences, influencing the pathological manifestations observed in rodents compared to humans.

Sheep models of HD

Although sheep are not commonly used in neurodegeneration research, they offer numerous advantages as experimental models, especially in the study of HD, due to their well-defined basal ganglia with distinct caudate and putamen nuclei, unlike rodents that lack these two distinct regions. Sheep models of HD have been developed by introducing human HTT cDNA into single-celled zygotes. Although this model expresses a low level of transgenic HTT and does not fully replicate the phenotypes seen in HD patients, it may serve as a suitable model for the prodromal or early stages of the disease (Jacobsen et al., 2010). HD sheep under the age of 5 do not exhibit noticeable movement symptoms, but they do show significant circadian rhythm abnormalities starting at 18 months old (Morton et al., 2014). Prior to the age of 5 years, there are no observable changes in brain volume or morphology in HD sheep, but mHTT-positive inclusions begin to appear in several cortical areas from 18 months onwards (Huntington's Disease Sheep Collaborative Research Group et al., 2013; Morton et al., 2014). These HD sheep models express the entire human HTT gene coding sequence, thus enabling the validation of drug effects and gene editing therapies.

A recent MRI study of HD sheep demonstrated that white matter alterations in the corpus callosum are associated with impaired gait (Taghian et al., 2022), consistent with findings from human HD studies. Additionally, markers of striatal energy metabolism in aged HD sheep correlate with a decline in clinical scores and impaired eye coordination. These results reaffirm the suitability of HD sheep as a valuable large animal model for preclinical assessment of HD therapies and, importantly, for noninvasive evaluation of therapeutic efficacy (Taghian et al., 2022). In a recent metabolomics study, Spick et al. (2023) observed significant absolute and rhythmic differences in metabolites between HD sheep at 5 and 7 years of age compared to age-matched control sheep. Notably, samples from 7-year-old HD sheep exhibited an increased number of altered metabolites and a greater shift in the acrophase compared to control sheep. Furthermore, the dysregulated metabolites identified in HD sheep showed remarkable similarity to those found in human patients, particularly involving phosphatidylcholines, amino acids, urea, and threonine (Morton, 2023; Spick et al., 2023).

Mears et al. (2021) integrated seven multiomics datasets from 5-year-old OVT73 (n = 6) and control (n = 6) sheep cohorts into a unified database using the R programming language. This comprehensive database encompasses high-throughput transcriptome, metabolomics, and proteomics data

Table 1 Summary of published Huntington's disease (HD) animal models

Animal model	Species	Construct	CAG repeat	HD-related phenotypes	Reference
R6/2	*Mus musculus*	5′UTR +exon 1 of human *HTT* +262 bp of intron 1	144	Aggregates: yes Neuron loss: yes Motor: yes	Hickey & Morton, 2000; Ona et al., 1999
R6/1	*Mus musculus*	5′UTR exon 1 of human *HTT* +262 bp of intron 1	116	Aggregates: yes Neuron loss: yes Motor: yes	Mangiarini et al., 1996
N118	*Mus musculus*	First 118 amino acids of human *HTT* cDNA	82	Aggregates: yes Neuron loss: no Motor: no	Tebbenkamp et al., 2011
N171	*Mus musculus*	N-terminal 171 amino acids of human *HTT* cDNA	82	Aggregates: yes Neuron loss: yes Motor: yes	Gardian et al., 2005; Schilling et al., 2004
HD94	*Mus musculus*	Tet-regulated system+ human/mouse exon 1+ intron 1	94	Aggregates: yes Neuron loss: yes Motor: yes	Yamamoto et al., 2000
Tg100 (TgCAG100)	*Mus musculus*	First 3 kb of human *HTT* cDNA	100	Aggregates: yes Neuron loss: yes Motor: yes	Laforet et al., 2001
HdhQ92	*Mus musculus*	Replacement of mouse Hdh exon 1 with human *HTT* with 92 CAG repeats	92	Aggregates: yes Neuron loss: yes Motor: yes	Brooks et al., 2012; Zhunina et al., 2019
HdhQ111	*Mus musculus*	Chimeric human/mouse exon 1	111	Aggregates: yes Neuron loss: yes Motor: yes	Wheeler et al., 2000; Zhunina et al., 2019
HdhQ150（CHL2）	*Mus musculus*	Expanded CAG in exon 1 of mouse *HTT*	150	Aggregates: yes Neuron loss: yes Motor: yes	Zhunina et al., 2019)
140Q	*Mus musculus*	Knock-in chimeric mouse/human exon 1 containing 140 CAG repeats inserted in murine huntingtin gene.	140	Aggregates: yes Neuron loss: yes Motor: yes	Menalled et al., 2003
zQ175	*Mus musculus*	Derived from spontaneous germline CAG expansion in *HTT* from CAG 140 line	179	Aggregates: yes Neuron loss: yes Motor: yes	Heikkinen et al., 2012
BAC226Q	*Mus musculus*	Full-length human *HTT* with ~226 CAG-CAA repeats	226	Aggregates: yes Neuron loss: yes Motor: yes	Shenoy et al., 2022
YAC72	*Mus musculus*	Full-length human *HTT*	72	Aggregates: yes Neuron loss: yes Motor: no	Hodgson et al., 1999; Seo et al., 2008
YAC128	*Mus musculus*	Full-length human *HTT*	125（interrupted by CAACAACAACAGCAA at positions 24–28 and 109–113）	Aggregates: yes Neuron loss: yes Motor: yes	Pouladi et al., 2012; Slow et al., 2003
BACHD	*Mus musculus and Rattus norvegicus*	Full-length human *HTT*	97	Aggregates: yes Neuron loss: yes Motor: yes	Gray et al., 2008; Yu-Taeger et al., 2012
HD 51	*Rattus norvegicus*	First 727 amino acids of rat *HTT* cDNA	51	Aggregates: yes Neuron loss: yes Motor: yes	Fink et al., 2012
Transgenic sheep	*Ovis aries*	Full-length human *HTT* cDNA encoding 73 polyglutamine repeats	73	Aggregates: yes Neuron loss: no Motor: no	Taghian et al., 2022
Injection monkeys	*Macaca fascicularis*	First 171 amino acids of human *HTT*	82	Aggregates: yes Neuron loss: no Motor: yes	Palfi et al., 2007
Transgenic monkeys	*Macaca mulatta*	Human *HTT* exon 1	84	Aggregates: yes Neuron loss: yes Motor: yes	Yang et al., 2008
Transgenic monkeys (second)	*Macaca mulatta*	Exons 1–10 of human *HTT* gene coding N-terminal 508 amino acids with approximately 67-72Q	67-72	Aggregates: yes Neuron loss: yes Motor: yes	Chan et al., 2015
Transgenic pig 1	*Sus domesticus*	First 1 100 amino acids of *Sus domesticus HTT* cDNA	75	Unclear	Uchida et al., 2001
Transgenic pig 2	*Sus domesticus*	First 208 amino acids of human *HTT* with 105Q (N208-105Q)	105	Aggregates: yes Neuron loss: yes Motor: yes	Yang et al., 2010
Transgenic pig 3	*Sus domesticus*	First 548 amino acids of human *HTT* cDNA fragment with stretch of 124 glutamines	124	Aggregates: yes Neuron loss: yes Motor: yes	Baxa et al., 2013
HD KI pig	*Sus domesticus*	Replacement of pig *HTT* exon 1 with human *HTT* exon 1 with 150 CAG repeats	150	Aggregates: yes Neuron loss: yes Motor: yes	Yan et al., 2018

obtained from blood, brain, and other tissues, effectively bridging the genetic gap between open-source HD mouse models and human data. Once relevant databases covering large animal models of HD are available, collaborative utilization and comparison of these resources will significantly enhance the depth and complexity of HD data for comparative molecular studies.

Non-human primate (NHP) models of HD

NHPs share many similarities with humans in terms of anatomy, physiology, genetics, cognition, emotion, and social behavior (Cui et al., 2022; Guo et al., 2023; Van Dam & De Deyn, 2017; Yao & Construction Team of the KIZ Primate Facility, 2022). Their nervous system is also comparable to that of humans, including the cerebral cortex and sulci, cortical neurons, prefrontal cortex, hippocampal projection pathways, neural control pathways, and blood-brain barrier structures (Edler et al., 2021; Li et al., 2023). Their brains are more similar in structure and development to humans than rodents, making them valuable for studying neurodegenerative diseases such as HD (Yin et al., 2022). The complete sequencing of the rhesus macaque (*Macaca mulatta*) and common marmoset (*Callithrix jacchus*) genomes was successfully achieved in 2007 and 2014, respectively. These genomic resources have been utilized for comparative analysis between species and for enhancing NHP models of disease (Emborg, 2017; Guo et al., 2023; Wu et al., 2022).

The first transgenic monkey model of HD was created using a lentiviral vector to deliver a transgenic fragment of human *HTT* exon 1 with 84 CAG repeats (Yang et al., 2008). Five macaques were born, three of whom carried multiple integration sites and higher mHTT transgene copy numbers. These positive monkeys were euthanized shortly after birth due to severe muscle tone disorders and respiratory distress (Yang et al., 2008). Although it remains unclear why the transgenic HD monkey models showed more severe phenotypes than mouse models, the phenotypic differences suggest that primate neurons may be more sensitive to HTT toxicity than rodent neurons. A second group of transgenic monkeys containing the first 11 exons of the human HTT gene bearing the first 508 amino acids and an expanded polyQ (67Q-72Q) was also established, exhibiting spontaneous motor dysfunction and epileptic seizures, as well as progressive cognitive decline. This model is closer to the chronic progression rather than acute progression of adult HD (Chan et al., 2015; Snyder & Chan, 2018). Both HD monkey models showed abundant HTT aggregates and axonal degeneration. Additionally, HD monkeys developed key clinical HD features including dystonia, chorea, and seizures (Yan et al., 2019). These findings suggest that large animals may be more sensitive to toxic HTT protein fragments than rodents. Therefore, it is tempting to think that expressing fragments of HTT protein in the brains of other large animals may also create valuable HD models.

However, there are several unresolved deficiencies in NHP models of HD that restrict their application. Firstly, monkeys have a long gestation period of approximately 6 months and are mostly born as singletons (Coe & Lubach, 2021). Additionally, somatic cell nuclear transfer (SCNT) technology in monkeys remains inefficient, making it challenging to obtain a large number of animals in a short period of time (Liu et al., 2018; Matoba & Zhang, 2018). Secondly, all biological experiments involving living subjects require ethical consideration. Given that NHPs are closely related to humans, research utilizing NHPs warrants particular attention. In practice, most institutional Animal Care and Use Committees (IACUCs) establish specialized review procedures to evaluate the scientific merit of experiments using NHPs, in addition to examining issues of animal safety and welfare stated in animal research proposals during regular project reviews (Estes et al., 2018). Moreover, the high cost of monkeys, as well as the expenses associated with their rearing and breeding under demanding conditions, continue to limit the widespread use of genetic NHP disease models (Tian, 2021).

Pig models of HD

Various large animal species have been used as models in biomedical research, with pigs showing many advantages. Firstly, pigs have similar neuroanatomy to humans, such as a prominent striatum divided into a separate caudate nucleus and putamen, which are not distinguishable in rodents (Pirone et al., 2023). Furthermore, pigs have several advantages over NHPs, including a relatively short gestation period (112–120 days), multiple offspring in a litter, short time to sexual maturity (6–7 months), and relatively easy and low-cost rearing. Thirdly, the availability of oocytes from slaughterhouses significantly reduces the cost of establishing disease models. Finally, SCNT technology is well-developed in porcines, especially when combined with CRISPR/Cas9 technology, paving the way for the establishment of precise gene-edited pig models (Ran et al., 2013; Yan et al., 2018).

As early as 2001, Uchida and colleagues attempted to establish a transgenic pig model of HD via prokaryotic injection, although whether these pigs exhibit the behavioral and neuropathological symptoms of the disease remains uncertain (Fan & Lai, 2013; Uchida et al., 2001). Later, Yang et al. (2010) successfully established a transgenic pig model of HD using SCNT methods, characterized by overexpression of the N-terminal mutant HTT (N208-105Q) and showing a postnatal lethal phenotype and abundant HTT aggregates, as well as apoptotic cells and chorea not found in transgenic HD mouse models. The striking differences between large and small transgenic HD animal models underscore the importance of using large animals to study HD neuropathology. These differences also indicate that overexpression of N-terminal mHTT is highly deleterious to large animals (Li & Li, 2015; Tu et al., 2015). Thus, establishing large animal models that express full-length mHTT at the endogenous level is important for investigating neuropathology and phenotypes.

Based on the above rationale, Yan and colleagues used pigs to successfully establish the first large animal model that endogenously expresses full-length mHTT. Using CRISPR/Cas9, they inserted a large CAG repeat (150Q) into the endogenous pig *HTT* gene in cultured fibroblast pig cells, then used SCNT to generate HD knock-in (HD-KI) pigs. The HD-KI pigs expressed full-length *mHTT* containing 150 CAG/Q repeats and showed age-dependent neurological symptoms, including body weight loss, early death, and movement difficulties. The brains of HD-KI pigs also showed the accumulation of HTT aggregates, similar to other HD animal models, and more importantly, displayed selective neurodegeneration in the striatum region, recapitulating an important pathological feature of HD patients. Furthermore, the pathophenotypes and neurodegeneration were transmittable through the germline, with F1 and F2 KI pigs

exhibiting similar pathophenotypes as the founder animals. The generation of HD-KI pigs demonstrates that large mammals can recapitulate overt and selective neurodegeneration and severe symptoms caused by mutant protein expressed at the endogenous level (Yan et al., 2018). Thus, HD-KI pigs could serve as an important tool to validate essential findings and therapeutic targets.

ANIMAL MODELS OF HD FOR TREATMENT

Considerable advancements in gene-editing technology have enabled the development of therapeutic strategies designed to repair, replace, or alter gene expression for the treatment of neurological diseases caused by single gene mutations (Sayed et al., 2022). These approaches, developed and validated through extensive research and clinical trials (Li et al., 2020), have enabled the identification of specific causative genes and mutations in several monogenic neurological disorders, providing the opportunity to develop disease-modifying therapies to change dysfunctional gene expression (Germain et al., 2023). Despite significant advances in our understanding of HD pathogenesis, effective treatments remain elusive. After decades of extensive research, the prevailing theory is that targeting and inhibiting the expression of expanded polyQ proteins offers a promising approach for treating such diseases (Wild & Tabrizi, 2017).

RNA interference (RNAi) technologies, like small interfering RNA (siRNA), microRNA (miRNA), and short hairpin RNA (shRNA), are being developed as potential therapies for neurological diseases. The most promising strategy is the use of antisense oligonucleotides (ASO) for inhibiting the expression of mHTT, demonstrating impressive therapeutic effects (Kordasiewicz et al., 2012; Tabrizi et al., 2019b). However, recent clinical failures have raised questions regarding the safety of HTT-lowering strategies in humans and the need to determine whether inhibiting mHTT expression alleviates neuropathology and symptoms of HD (Tabrizi et al., 2019a). Therefore, the strategy of directly replacing mutated gene segments is an attractive potential approach for treating monogenic inherited diseases.

Treatment can be achieved through a single brain stereotaxic injection or intravenous delivery of CRISPR/Cas9 with partial replacement of m*HTT* (Yan et al., 2023). Intravenous delivery of CRISPR/Cas9 is typically performed in newborn animals before the blood-brain barrier is fully established. This allows for systemic reduction of HTT toxicity in both the brain and peripheral tissues. Conversely, brain stereotaxic injection provides a more targeted effect on specific brain regions, which is advantageous for alleviating striatum-associated phenotypes in adult animals. The therapeutic effects of CRISPR/Cas9 on large animal models of neurodegenerative diseases highlight its great potential in the treatment of neurological disorders in humans.

PERSPECTIVES

Large animal models are not only instrumental in advancing gene therapy for HD but also provide the means to address key aspects of disease treatment. Notably, these models can aid in identifying reliable early pathological biomarkers and defining specific biomarkers that can characterize disease progression. These models can also be applied to address several critical questions. For example, do HTT, mHTT, and other key proteins interact, and what is the mechanism underpinning mHTT aggregation? What roles do normal HTT and mHTT play throughout the entire pathological process in HD patients? Beyond gene therapy, are small molecule or antibody drugs that directly target proteins effective, and at what stage should such targeted protein therapy be introduced?

However, all current animal models of HD exhibit certain limitations. A primary constraint is the absence of large animal models that express the complete human *HTT* gene sequence, including introns, 5' and 3' untranslated regions (UTRs), and CAG expansions. This lack of complete genetic representation hinders our understanding of the comprehensive pathological process and poses a challenge to gene therapy development. Additionally, in many HD animal models, especially in pigs, longer CAG repeat lengths may be required to sustain significant disease, which is less common in human cases. Furthermore, when introducing 39–50 CAG repeats similar to those in adult-onset HD patients, model animals may not develop pronounced disease symptoms within their relatively short 10–20-year lifespans. Finally, while genome-editing techniques, such as CRISPR-Cas9, have revolutionized the field of animal modeling, including in HD research, they also exhibit certain limitations. A notable concern is the possibility of off-target effects, leading to unintended genomic alterations. Furthermore, CRISPR/Cas9 may also cause mosaic effects, which can be particularly significant in large animals and in studies requiring the use of founders. These limitations have the potential to confound the phenotypes observed in generated animal models.

Despite their challenges, large animal models of HD have provided unique insights into disease pathogenesis. Furthermore, the successful use of large animals as models for HD highlights their significance for other neurodegenerative disorders. While rodent models offer valuable insights into the mechanisms of neurodegeneration, large animal models can also play a crucial role in validating fundamental discoveries and therapeutic targets.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

B.H., W.L., and Z.T. wrote the original draft. X.J.L., S.L., and S.Y. conceptualized, wrote, and edited the manuscript. All authors read and approved the final version of the manuscript.

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