# Influence of Species Differences on the Neuropathology of Transgenic Huntington's Disease Animal Models

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# Influence of Species Differences on the Neuropathology of Transgenic Huntington's Disease Animal Models

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## Abstract

Transgenic animal models have revealed much about the pathogenesis of age-dependent neurodegenerative diseases and proved to be a useful tool for uncovering therapeutic targets. Huntington's disease is a well-characterized neurodegenerative disorder that is caused by expansion of a CAG repeat, which results in expansion of a polyglutamine tract in the N-terminal region of huntingtin (HTT). Similar CAG/glutamine expansions are also found to cause eight other neurodegenerative diseases that affect distinct brain regions in an age-dependent manner. Identification of this CAG/glutamine expansion has led to the generation of a variety of transgenic animal models. Of these different animal models, transgenic mice have been investigated extensively, and they show similar neuropathology and phenotypes as seen in their respective diseases. The common pathological hallmark of age-dependent neurodegeneration is the formation of aggregates or inclusions consisting of misfolded proteins in the affected brain regions; however, overt or striking neurodegeneration and apoptosis have not been reported in most transgenic mouse models for age-dependent diseases, including HD. By comparing the neuropathology of transgenic HD mouse, pig, and monkey models, we found that mutant HTT is more toxic to larger animals than mice, and larger animals also show neuropathology that has not been uncovered by transgenic mouse models. This review will discuss the importance of transgenic large animal models for analyzing the pathogenesis of neurodegenerative diseases and developing effective treatments.

### Keywords

## 1. INTRODUCTION

Several important neurological disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD), share a common pathological feature:

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selective neurodegeneration that is caused by misfolded proteins and occurs in an age-dependent manner. Strong evidence for protein misfolding in this type of disease is the presence of protein aggregates in the brains of affected patients. For example, in the brains of patients with AD, extracellular aggregates (senile plaques), which are formed by beta amyloid (Aβ) proteins, are the pathological hallmark of AD, whereas Lewy bodies, which contain α-synuclein, are a characteristic brain pathology of patients with PD. In HD, nuclear inclusions and neuropil aggregates are formed by N-terminal mutant huntingtin fragments that carry more than 36 polyglutamine repeats. Although whether these aggregates are toxic or protective is still a hot topic of debate, it is clear that these aggregates are formed by small misfolded peptides and reflect the accumulation of misfolded proteins. Consistently, the age-dependent formation of these aggregates in patient brains is correlated with the progression of neurological symptoms in AD, PD, and HD.

The common pathological changes in these neurodegenerative diseases point to the critical role of protein misfolding in mediating neurological phenotypes; however, these diseases affect distinct types of neuronal cells despite the widespread expression of the disease proteins. For example, AD mainly affects the cortical neurons, whereas PD selectively affects dopaminergic neurons in the brain region known as the substantia nigra. HD, on the other hand, preferentially affects the medium spiny neurons in the striatum. The selective neuropathology in each disease indicates that protein context is important for the specific neuronal toxicity in these diseases. Because all these disease proteins have different functions and interact with different partners, protein misfolding is likely to alter their functions and association with other proteins, resulting in a gain of toxicity in specific types of neurons. Indeed, a large number of studies have demonstrated that mutant huntingtin (HTT) can abnormally interact with a variety of proteins and alters their cellular functions (Harjes and Wanker, 2003; Li and Li, 2004). Importantly, unlike the majority of AD and PD cases, which are sporadic, HD is an inherited neurological disease caused by a single gene mutation. Thus, HD provides an ideal system to investigate pathogenesis caused by misfolded proteins. The mechanistic insight obtained from studying HD would be very helpful for understanding other neurodegenerative diseases, such as AD and PD, which are also caused by protein misfolding. Thus, this review will focus on HD and its animal models.

## 2. HD AND POLYGLUTAMINE DISEASES

HD is an autosomal dominant genetic neurological disorder characterized by motor dysfunction, cognitive decline, and psychological dysfunction. HD displays selective neurodegeneration that occurs preferentially in the brain striatum (Gusella et al., 1993; Vonsattel and DiFiglia, 1998). The majority of patients with HD show symptoms in midlife and often die 10–15 years after the onset of symptoms. The genetic cause of HD is the expansion of a CAG repeat (>36 CAGs) in exon1 of the HD gene (*HTT*). Thus, the CAG repeat expansion results in an expanded polyglutamine (polyQ) tract in the N-terminal region of *HTT*, a large-sized protein (3144 amino acids) that is ubiquitously expressed in various types of cells and interacts with a number of proteins (Harjes and Wanker, 2003; Li and Li, 2004). It is known that the CAG repeat is unstable and its length varies in different species (Matsuyama et al., 2000). However, the expansion of this repeat in different polyQ

disease genes can lead to abnormal protein conformations, such as a β-sheet structure (Perutz et al., 1994). As a result, mutant HTT with an expanded polyQ tract forms insoluble aggregates or inclusions in the brains of patients with HD in an age-dependent manner.

There are eight other inherited neurodegenerative diseases that are also caused by an expansion of a polyQ tract, including spinocerebellar ataxia (SCA) types 1–3, 6, 7, and 17, spinobulbar muscular atrophy (SBMA), and dentatorubropallidoluysian atrophy (DRPLA) (Orr and Zoghbi, 2007). These polyQ disease genes do not share homology, except for the CAG/polyQ stretch; however, all the polyQ diseases share many common pathological features. First, symptoms in these diseases usually appear at midlife and progressively worsen until death, some 15–20 years later. Longer CAG/polyQ repeat sizes are associated with an earlier age of disease onset. Second, these diseases all show aggregates or inclusions formed by mutant proteins in the brain, a pathological hallmark of the polyQ diseases. Third, the expression of these mutant proteins is generally widespread throughout the body, but selective degeneration is seen in specific regions of the central nervous system.

HD is the most common polyQ disorder that has been well characterized for its selective neurodegeneration. The selective neurodegeneration in HD occurs early in the medium spiny neurons in the striatum. Other brain regions, such as the deep layers of the cortex, the hypothalamus, and the hippocampus, also undergo neurodegeneration in the later stages of HD (Vonsattel and DiFiglia, 1998). While the primary function of HTT has yet to be determined, it is known to be essential for early development and probably plays a role in cellular trafficking as a scaffold protein (Harjes and Wanker, 2003; Li and Li, 2004). It is evident that only N-terminal mutant HTT is able to form aggregates and is more toxic than full-length mutant HTT (Gutekunst et al., 1999; Zhou et al., 2003), which has led to extensive studies to identify the proteolytic cleavage sites mediated by various proteases, including calpains, aspartyl proteases, and caspases, which can degrade mutant HTT to generate N-terminal HTT fragments (Qin and Gu, 2004). In addition to having the abilities to misfold and aggregate, N-terminal mutant HTT fragments can also accumulate in the nucleus, whereas the majority of full-length mutant HTT remains in the cytoplasm (DiFiglia et al., 1997; Gutekunst et al., 1999). The nuclear localization of N-terminal mutant HTT can lead to abnormal binding of mutant HTT to various transcription factors, subsequently affecting transcriptional expression (Harjes and Wanker, 2003; Li and Li, 2004).

## 3. MOUSE MODELS OF HD

Identification of the genetic mutation for HD has led to the establishment of various transgenic HD mouse models. These models include transgenic mice (R6/2 and N171-82Q) expressing N-terminal mutant HTT (Davies et al., 1997; Schilling et al., 1999), full-length mutant HTT transgenic mice (YAC and BAC) (Slow et al., 2003; Gray et al., 2008), and HD repeat knock-in (KI) mice (Wheeler et al., 2000; Lin et al., 2001; Menalled et al., 2002). R6/2 and N171-82Q mice display abundant HTT aggregates in their brains at 3–4 months, as well as severe neurological symptoms and earlier death at 3–6 months (Davies et al., 1997; Schilling et al., 1999). Yeast artificial chromosome transgenic mice (YAC128), HD KI mice, and BAC-HD transgenic mice, which express full-length mutant human HTT with an expanded polyQ repeat (114-150Q), display obvious HTT aggregates only at older ages (7–

10 months), show milder neurological symptoms than R6/2 and N171-82Q mice, and can survive as wild-type mice (Lin et al., 2001; Slow et al., 2003; Gray et al., 2008; Wang et al., 2008). A careful analysis of HD150Q KI mice at 22 months, however, demonstrated that they develop the well-characterized HTT aggregates as seen in R6/2 mice at the age of 12 weeks (Woodman et al., 2007). Thus, characterization of various HD mouse models provides clear evidence that small N-terminal HTT fragments with expanded polyQ tracts become misfolded and form aggregates. Consistently, polyQ-containing N-terminal HTT fragments also form aggregates in HD cellular models (Li and Li, 1998) and in HD patient brains (DiFiglia et al., 1997; Gutekunst et al., 1999). Despite the milder phenotypes of HD mice that express full-length mutant HTT, such as YAC128 and HD KI mice, these mice show preferential accumulation of mutant HTT in the striatum, consistent with the preferential loss of the medium spiny neurons in the striatum of HD patients. Thus, the accumulation of mutant HTT in neuronal cells is clearly a prerequisite for neuronal dysfunction and degeneration.

Although HD mouse models have been used widely to uncover the pathogenesis of HD and to develop treatments, most of these mouse models show no apoptosis or overt neurodegeneration in their brains. Similarly, in other polyQ mouse models, the lack of striking neurodegeneration is also a noteworthy phenomenon. Further, transgenic mice for AD and PD do not show typical neurodegeneration, either (Dawson et al., 2010), although neurodegeneration is the major pathological event in AD and PD patients (Mattson, 2000; Yuan and Yankner, 2000). All these facts point out that, although mouse models are used widely to investigate the pathogenesis of HD and other neurological diseases, they have their limitations and do not replicate the full range of neurological phenotypes seen in human diseases. Such limitations reflect the importance of species differences in the development of neurodegeneration.

## 4. SPECIES DIFFERENCES AND APPROACHES FOR GENERATING TRANSGENIC LARGE ANIMALS

Considering that HD and other neurodegenerative diseases are age-dependent disorders, it is important to understand the differences in the aging process among different animal species when these animals are used to model neurodegenerative diseases. Table 1 summarizes several important biological aspects of different species (mouse, pig, monkey, and human). The lifespans of these species differ drastically, indicating that aging processes in different species are not identical. Also, the early development of these mammalian species requires significantly different periods of time. For example, the gestation period for mice is 21 days, whereas pigs and monkeys require 4–5 months to reach full-term development. In addition to these significant differences, the anatomy, physiology, function, and circuitry of pig and monkey brains are much more complex than mouse brains. These differences clearly indicate that monkeys and pigs are much closer to humans than mice and also explain why larger animal models would be better to mimic the pathological features seen in human patients. Indeed, a genetic pig model of cystic fibrosis replicates abnormalities seen in cystic fibrosis patients that do not occur in mouse models (Rogers et al., 2008). Of the large animals, monkeys are the best to model neurological diseases of humans, especially for

cognitive behavioral analysis. Pigs, on the other hand, have a long lifespan (12–15 years), are easily bred, and reach puberty at 5–6 months, so they also offer advantages for biomedical research over other large animals, such as primates, for ethical and economic reasons (Lind et al., 2007).

Nevertheless, generation of genetic models using large animals is much more challenging than establishing genetic mouse models. To generate genetic mouse models, we can use established pronuclear injection to integrate transgenes into the chromosomes of germline cells of mice. We can also use mouse embryonic stem (ES) cells to perform gene targeting, which allows us to alter the expression of the endogenous genes. So far, there are no ES cells from pigs, monkeys, or other large animals that can be used for generating gene-targeted animals. Induced pluripotent stem (iPS) cells are similar to embryonic cells and can be potentially used for altering endogenous genes and gene targeting in various species of animals. However, use of iPS cells for gene targeting is still under development. Most work done on transgenic monkeys involved the use of lentiviral vector infection of fertilized oocytes and embryo transplantation (Yang et al., 2008; Sasaki et al., 2009; Niu et al., 2010), which requires a considerable number of donor and surrogate monkeys. Successful generation of transgenic pigs can also be achieved *via* nuclear transfer, a cloning strategy that has a low rate (<1%–2%) for transferred pig embryos to develop to term (Lai and Prather, 2003). In addition, the costs in maintaining and breeding large animals as well as the ethical concerns and strict regulation of the use of large animals also make it difficult to use them for biomedical research. Despite these disadvantages, the closer similarities of biological functions between large animals and humans clearly indicate the importance of using pigs and monkeys to give us pathogenic insights that cannot be gleaned from mouse and other small animal models.

## 5. THE NEUROPATHOLOGY OF TRANSGENIC HD MONKEYS AND PIGS

Although biological differences between humans and mice may account for the failure of some mouse models to replicate pathology in humans, whether larger transgenic animal models can mimic important neurodegenerative features caused by misfolded proteins remains to be rigorously tested. The creation of a transgenic monkey in 2001 (Chan et al., 2001) demonstrated that the monkey genome could be genetically modified and has led to the generation of transgenic nonhuman primates expressing disease genes or exogenous foreign genes (Yang et al., 2008; Sasaki et al., 2009; Niu et al., 2010). Our collaboration with Dr. Anthony Chan at Emory University in Atlanta, USA, has led to the development of transgenic HD rhesus monkeys that express exon1 mutant *HTT* with 84Q under the control of the human ubiquitin promoter (Yang et al., 2008). These HD monkeys were generated by injecting lentiviruses into fertilized oocytes to express mutant HTT. Unlike transgenic mice, which can survive after birth when expressing the same exon1 mutant HTT with an even longer polyQ repeat (150Q), HD transgenic monkeys with 84Q could die postnatally and this early death is associated with the levels of mutant HTT (Yang et al., 2008). However, these transgenic monkeys developed key clinical HD features, including dystonia, chorea, and seizure (Yang et al., 2008), which have not been replicated by mouse models or other small animal models. Like the brains of HD mouse models and patients, the HD monkey brains also show abundant HTT aggregates in the neuronal nuclei and neuronal processes

(Fig. 1A). More importantly, the transgenic HD monkeys display degeneration of axons and neuronal processes in the absence of obvious cell body degeneration, suggesting that neuronal degeneration in HD may initiate from neuronal processes (Fig. 1B). Such findings provide us with valuable information to understand the pathogenesis of HD.

Our collaboration with Dr. Liangxue Lai at the Guangzhou Institutes of Biomedicine and Health (GIBH), Chinese Academy of Sciences, also led to the generation of transgenic HD pigs that express N-terminal mutant HTT consisting of the first 208 amino acids with 105Q (N208-105Q) (Yang et al., 2010). The transgenes were expressed under the control of the cytomegalovirus enhancer and chicken *β-actin* (CAG) promoter to allow for the ubiquitous expression of transgenes in all tissues. Primary porcine fetal fibroblast cells expressing this mutant HTT were used to generate transgenic HD pigs *via* nuclear transfer. Six early pregnancies were established, and four of them went to term, with five live births. However, most of these transgenic HD piglets die postnatally, and some transgenic HD pigs show a severe chorea phenotype before death. We also generated transgenic mice expressing the same mutant HTT and found that transgenic HD mice could live up to 9 months. Thus, the postnatal death of transgenic HD piglets also suggests that mutant HTT is more toxic to larger animals. More importantly, in all transgenic pig brains examined, there were apoptotic cells (Fig. 2, Yang et al., 2010), which have not been reported in any HD mouse models.

The generation of transgenic HD monkeys and pigs provides further evidence for the toxicity of N-terminal mutant HTT. In support of this idea, transgenic HD sheep expressing full-length mutant HTT with a 73Q tract live normally and show only a decrease in the expression of the medium spiny neuron marker DARPP-32 (Jacobsen et al., 2010). Thus, as with HD mouse models, the expression of N-terminal mutant HTT can cause robust neurological phenotypes and pathological changes in large animals. These studies also suggest that protein context and the length of HTT fragments may determine the nature of the neuropathology. For example, exon1 (1–67 amino acids) mutant HTT in monkey brains causes axonal degeneration, whereas N-terminal 208 amino acids of mutant HTT in pig brains can mediate apoptosis; however, in a transgenic pig (Uchida et al., 2001) expressing a larger mutant HTT fragment (1100 amino acids) and in transgenic HD sheep (Jacobsen et al., 2010) expressing full-length (3144 amino acids) mutant HTT, there was no apoptosis, early animal death, or neurological phenotype reported. It is possible that neurodegeneration in large animals only occurs when sufficient degraded N-terminal fragments have accumulated in old animals. Thus, expressing N-terminal mutant HTT fragments can facilitate disease progression, resulting in the early postnatal death of transgenic HD pigs and monkeys.

## 6. INSIGHTS FROM ANALYZING TRANSGENIC HD MONKEYS AND PIGS

By comparing the neurological symptoms and neuropathology of transgenic HD mice, pigs, and monkeys, it is clear that species differences play a critical role in the neurological phenotype differences in these animal models. An interesting issue is what the mechanisms behind these differences are. Certainly, there are a number of possible explanations. The short lifespan of mice is often believed to be responsible for the failure of HD mouse models to develop overt neurodegeneration. It is also possible that the misfolded form of N-terminal

mutant HTT is more toxic to the neuronal cells of pigs and monkeys than to rodent neurons. Considering that gestation in monkeys and pigs is much longer than in mice, this longer time period may allow overexpression of the toxic form of mutant proteins, such as N-terminal mutant HTT, to cause more severe neurotoxicity in the pig and monkey brains. Also, because the brain circuitry in pigs and monkeys is more complex than in mice, this complexity may make neurons in large animals more vulnerable to misfolded mutant HTT. Finally, the cellular ability to cope with misfolded proteins during development and adulthood may be different between species. The rapid maturation of rodent neurons during early brain development may reduce their sensitivity to misfolded proteins, which can also explain why mouse models can survive to adulthood even when they express the same mutant HTT N-terminal fragment.

The above possibilities underscore the importance of studying transgenic large animal models. For example, it would be interesting to know how mutant HTT possesses neuronal toxicity in adult transgenic animals. Such studies would require new transgenic animal models that can survive to adulthood, which can be accomplished by using different transgenic vectors that express mutant HTT at a lower level or in an inducible manner. To verify that N-terminal mutant HTT, rather than its overexpression, is indeed toxic, it would also be important to use a knock-in approach to express N-terminal mutant HTT at the endogenous level. Such an experiment could be done using pigs, as gene targeting is feasible in cultured pig fibroblast cells that will be used for the nuclear transfer. Transgenic models using higher mammalian species or large animals to model important neurodegenerative diseases would give us deeper insight into the pathogenesis of neurodegenerative diseases.

## 7. CONCLUDING REMARKS

By comparing different transgenic animal models for HD, this review has described some important differences in neuropathology that may be caused by species differences. Thus, larger animal models would be an important tool for us to further untangle the pathology of these diseases; however, we must point out that, because of the difficulty and expense of generating and characterizing large animals, the rodent models of neurodegenerative diseases will remain as a major modeling system for investigating a variety of diseases. For some critical pathological changes found in small animal models, large animal models will make a more rigorous system for validating the relevance of these findings to human diseases. In addition, given the frequent failures when it comes to clinical trials of drugs that have been found to work in small animal models, transgenic large animals could yield a more reliable system for verifying therapeutic efficacy before the step of clinical trials.

## Acknowledgments

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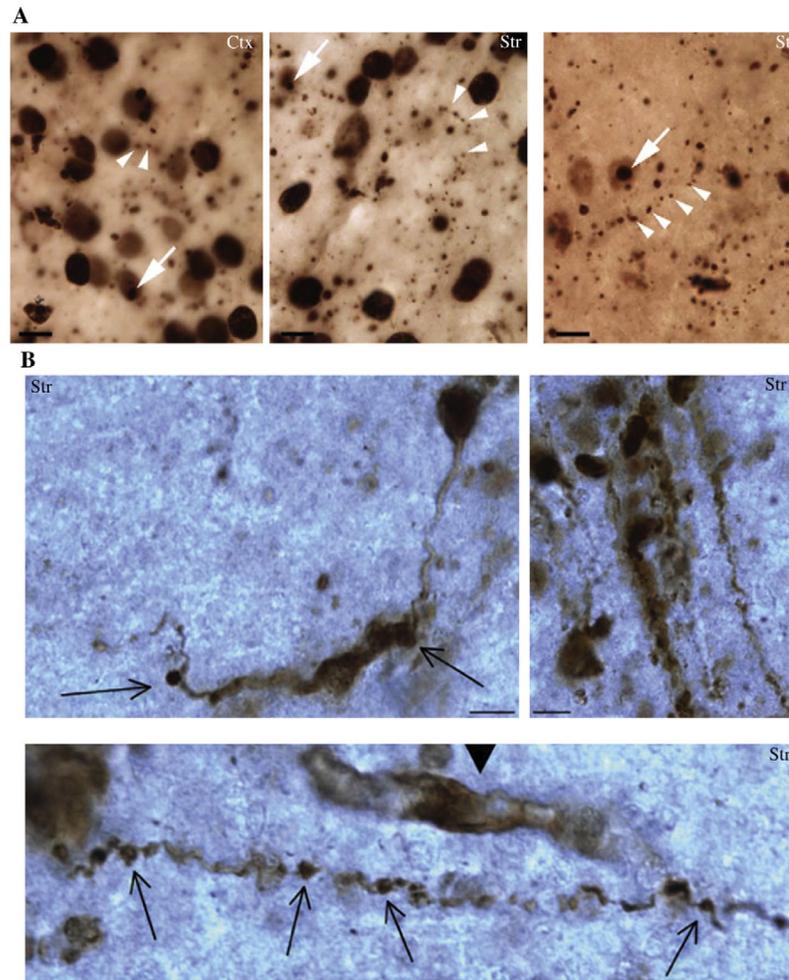
**Fig. 1. Axonal degeneration in transgenic HD monkey brains**
**A:** HTT (EM48) immunostaining showing the presence of HTT aggregates (arrows) in the neuronal nuclei in the cortex and striatum and in the neuronal processes (multiple arrowheads). **B:** HTT immunostaining also revealed axonal degeneration (arrows) in HD monkey brain. Arrowhead indicates a glial cell. Ctx, cortex; Str, striatum. See Wang et al., 2008, Hum. Mol. Genet., published by Oxford University Press.
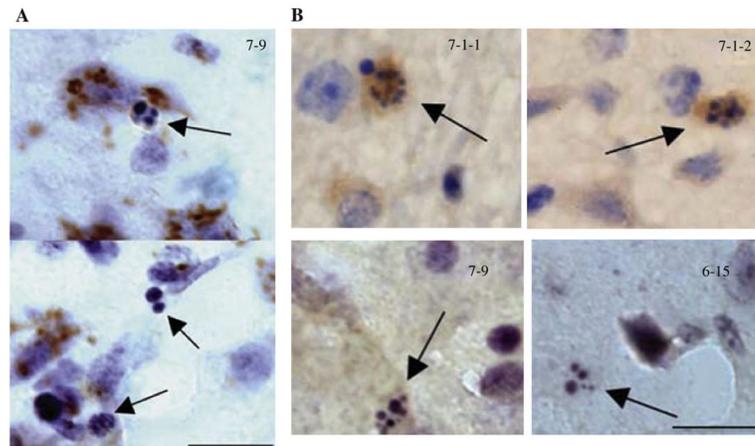
**Fig. 2. Apoptotic cells in the brains of transgenic HD pigs**
**A:** anti-polyQ (1C2) immunocytochemistry revealed the presence of mutant HTT in the neurons of the brain striatal (upper) and cortical (lower) sections of HD transgenic pig (7-9).
**B:** HTT (EM48) immunocytochemistry also revealed apoptotic neurons (arrows) in transgenic HD pigs (7-1-1, 7-1-2, 7-9, and 6-15). Scale bars: 10 μm. See Yang et al., 2010, Hum. Mol. Genet., published by Oxford University Press.

**Table 1**

Major differences in some species

| Species | Sex maturity | Gestation period (day) | Lifespan (year) | Body weight (kg) |
|---|---|---|---|---|
| Human | 15–18 years | 266 | 75 | 50 |
| Monkey (Rhesus) | 3–5 years | 165 | 25 | 6 |
| Pig | 9–11 months | 114 | 12–15 | 80 |
| Mouse | 7 weeks | 19–21 | 2 | 0.03 |