Choosing an animal model for the study of Huntington’s disease

Mahmoud A. Pouladi¹,², A. Jennifer Morton³, Michael R. Hayden¹,²,⁴,⁵,*

¹ Translational Laboratory in Genetic Medicine, Agency for Science, Technology and Research,
² Department of Medicine, National University of Singapore, Singapore 117609,
³ Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1PD, UK,
⁴ Centre for Molecular Medicine and Therapeutics, Child and Family Research Institute, University of British Columbia, Vancouver, Canada V5Z 4H4, and
⁵ Teva Pharmaceutical Industries Ltd, 16 Basel St, Petah Tikva, Israel, 49131
* Corresponding author: mrh@cmmt.ubc.ca

Since the identification of the causative gene in Huntington disease (HD), a number of animal models of this disorder have been developed. A frequently asked question is: which of these models most closely recapitulates the human disease? In this Review, we provide an overview of the currently available animal models of HD, in the context of the clinical features of the disease. In doing so, we highlight their strengths and limitations for modelling specific symptoms of the disease. This should highlight the animal model that is best suited to address a particular question of interest and, ultimately, to expedite the discovery of treatments that will prevent or slow the progression of HD.

Introduction
Huntington disease (HD) is a fatal progressive neurodegenerative disorder¹. It is inherited in an autosomal dominant fashion and is caused by a polymorphic trinucleotide CAG repeat expansion in exon 1 of huntingtin (HTT). Expansion of the CAG tract (that codes for polyglutamine) to greater than 36 repeats results in disease. Longer CAG repeat tracts accelerate the onset of the disease, which appears to be influenced, albeit to a lesser extent, by environmental and genetic
modifiers. The clinical hallmarks of HD include a triad of cognitive, psychiatric and motor disturbances as well as peripheral phenotypes including weight loss and muscle wasting.

Since the discovery of the \( HTT \) mutation 20 years ago, close to 10,000 papers have been published on HD, approximately half of which relate to attempts to model various aspects of the disease. The organisms used for this endeavour have included worms (\( Caenorhabditis elegans \)), fruit flies (\( Drosophila melanogaster \)), mice, rats, sheep and, more recently, pigs and monkeys. The findings from animal model studies have helped elucidate important pathways that are disrupted in HD and have provided important insights into the pathogenesis of this disease. These developments have been accompanied by the identification of several therapeutic candidates and novel approaches to therapy\(^2\). Indeed, over 15 clinical trials have been conducted in patients with HD since the discovery of the \( HTT \) mutation\(^1,3\).

Despite the considerable progress in our understanding of the disease, an effective treatment that either prevents or slows the pace of HD remains out of reach. The slow rate of progression of HD often necessitates lengthy and therefore costly clinical trials. In addition, the number of patients with HD that are available to participate in such trials is limited, which means that a relatively small number of compounds can be tested at any one time. Thus, animal models will continue to have a role not only as a filter for test compounds prior to the initiation of human clinical trials but also as a means for identifying candidate compounds with therapeutic promise.

The translational potential of an animal model of disease may be gauged on the basis of its construct, face and predictive validity (BOX 1). In this Review, we aim to provide an overview of the progress made in modelling HD in various animal species from the perspective of the clinical symptoms of HD, highlighting their genetic (construct) and phenotypic (face) characteristics. We focus on fruit fly, rodent, pig, sheep and monkey models of HD. Understanding the strengths of the various model systems, along with knowledge of the phenotypes and endpoints that have been characterized in these models, should aid in the
selection of the right animal model for a particular purpose and question of interest. In addition, by highlighting aspects of the clinical phenotype of HD that remain unexamined in animal models, we hope to illuminate relevant areas for potential future disease-modelling efforts.

**Early animal models**

Before the discovery of the genetic mutation underlying HD, models of this disease relied on neurotoxin-mediated striatal lesioning\(^4\)\(^-\)\(^6\). The basis for these lesional models was the observation that the primary site of neurodegeneration in HD was the striatum, and that intrastral injections of glutamate receptor agonists resulted in selective loss of the GABAergic projection neurons that are most severely affected in this disorder. The first neurotoxin models used ibotenic acid and kainic acid. These compounds were supplanted by quinolinic acid, because lesions caused by quinolinic acid (relatively) spared striatal interneurons, which are largely unaffected in HD\(^4\)\(^-\)\(^9\). In addition to glutamate agonists, peripheral administration of mitochondrial toxins such as malonate and 3-nitropropionic acid (3-NPA) were used to generate lesion models\(^10\)\(^,\)\(^11\). These toxins cause cellular energy depletion by targeting the electron transport chain, and chronic treatment with them resulted in the spontaneous formation of bilateral lesions, predominantly in the striatum, that also showed relative sparing of striatal interneurons\(^10\)\(^,\)\(^11\). Mitochondrial toxin-induced striatal lesions were also used as acute models of HD in rodents and non-human primates\(^9\)\(^,\)\(^11\)\(^-\)\(^13\).

The excitotoxic lesion models of HD were valuable in highlighting potential pathways and processes that were subsequently implicated in the pathogenesis of HD, including aberrant NMDA-type glutamate receptor-mediated signalling\(^14\)\(^-\)\(^16\), and mitochondrial dysfunction and associated energetic defects\(^17\)\(^-\)\(^21\).

Nevertheless, these models have a number of limitations, including the acute nature of the lesions. Even striatal lesions induced by chronic 3-NPA treatment develop quickly, taking mere days to appear. This scenario contrasts sharply with the slow and protracted pace of HD, which takes decades to manifest. Thus, progressive, age-dependent pathogenic events cannot be represented in the
acute lesion models. Furthermore, although striatal atrophy is indeed the cardinal neuropathological feature of HD, mutant HTT (mHTT) is expressed throughout the CNS and is widely expressed in peripheral tissues. Extrastral as well as peripheral pathologies resulting from this widespread pattern of mHTT expression are likely to contribute to the core pathological features of the disease. Thus, acute striatal lesion-based models do not lend themselves to the study of these aspects of the disease. Finally, the restricted pathology caused by striatal lesions means that many of the neurological manifestations of HD that are caused by pathology in other parts of the brain are not reproduced in the lesion models. The lesion models have been well reviewed in the past and in view of their limited usefulness, they will not be discussed further. Instead, we will focus on genetic animal models of HD.

**Genetic features of animal models**

HD is caused by a dominant mutation in a single gene. Theoretically, this should make the generation of genetic models very easy. However, both the characteristics of HTT and the nature of the mutation have made working with this gene very challenging (BOX 2).

A number of genetic approaches have been used to generate animal models of HD. The key distinguishing factors between these approaches include the following: use of full-length or only a fragment of mutated HTT; the length of the CAG repeat incorporated into the genetic construct; use of a coding region containing only CAG repeats or one containing repeats that are interrupted with one or more CAA codons (which also code for glutamine); the expression of the HD mutation from a transgene or knock in of the mutation into the endogenous Hdh locus; use of human HTT or the endogenous gene from the animal; use of complementary DNA (cDNA) or genomic DNA containing all the introns and regulatory sequences; and use of the HTT promoter or another promoter to drive expression of the mutant protein (FIG. 1).
**C. elegans models.** The transgenic *C. elegans* models of HD established to date express truncated N-terminus fragments of mHTT ranging from 57 to 171 amino acids in targeted neurons^{24,25} (TABLE 1). Despite the absence of a *HTT* orthologue in *C. elegans*, transgenic expression of mHTT in worms results in age-dependent mechanosensory defects, neuronal dysfunction and, on a sensitizing background, neurodegeneration^{24,25}.

**D. melanogaster models.** With the exception of one study^{26}, *D. melanogaster* models of HD have made use of the upstream activating sequence (UAS)-GAL4 systems^{27} to drive the expression of full-length or truncated N-terminal fragments (ranging in size from 65 to 548 amino acids^{26,28-32}) of mHTT in targeted cellular populations (TABLE 1). Note that the full-length mHTT models express cDNA constructs, which therefore lack *HTT* introns^{33} and as such would not recapitulate potential pathogenic events attributed to intronic features, such as generation of a *HTT* exon 1 fragment due to the presence of a cryptic poly-adenylation signal in intron 1 of *HTT*^{34}. Overall, the HD fly models exhibit a progressive degenerative phenotype along with motor abnormalities and reduced survival.

**Rodent models.** Rodents are by far the most commonly used animals for modelling HD. Indeed, over 20 different rodent models of this disease have been generated to date (TABLE 2). Here, we focus on the most commonly used ones that also represent different approaches in genetic design. The R6/1, R6/2 and N171-Q82 (referred to as N171 from here on) mouse models of HD express truncated N-terminal fragments of mHTT. R6/1 and R6/2 mice express exon 1 of human *HTT*, originally with 116 and 144 CAG repeats respectively, under the control of the human *HTT* promoter^{35}. Somatic instability of the CAG repeat tract has been observed in R6/1 and R6/2 mice^{36}. In the case of the R6/2 mice, the expanded CAG repeat tract was also found to be unstable in the germ cells, and transmissible CAG repeat expansions >400 CAGs have been reported^{37,38}. The instability of the CAG tract and associated improvement in reproductive viability
in R6/2 mice means that in many colonies, the size of the expansion in R6/2 mice is greater than the size that was originally reported. Indeed, unless it is otherwise stated in a manuscript, it should be assumed that the size of the expansion in R6/2 mice is 200–250 CAGs. There are now two R6/2 mouse colonies that are available from Jackson Laboratories: one has an expansion of 110 repeats, whereas the other has an expansion of 250 repeats. N171 mice express a truncated HTT cDNA with 82 CAG repeats under the control of mouse PrP promoter. The truncated N-terminal fragment models all typically exhibit a rapid onset of symptoms, including motor, cognitive and behavioural abnormalities, weight loss and a reduction in lifespan. These symptoms are accompanied by a widespread and generalized degenerative phenotype.

Full-length mHTT rodent models were established either by ‘knocking in’ a human HTT exon 1 with an expanded CAG repeat tract into the endogenous mouse Hdh gene locus, or by the introduction of a full-length mutant human HTT transgene (TABLE 2). Knock-in mice carrying a CAG tract-expanded Hdh with CAG repeat sizes ranging from 50 to 200 have been generated. For the purpose of this Review, we focus on three knock-in models, the HdhQ111, CAG140 and HdhQ150 mice. These models are genetically precise, carrying one or two copies of the mutant HD gene at endogenous levels and result in temporally and spatially appropriate levels of mHTT expression. The HdhQ111 mice show germ line and somatic repeat instability. Somatic instability of the CAG repeat tract has also been observed in HdhQ150 animals. In addition to difference in the length of the CAG tract, the full-length knock-in models differ in the nature of their exon 1 proline-rich region: HdhQ150 has the mouse proline-rich region, whereas the HdhQ111 and CAG140 mice have the human proline-rich region. Knock-in mice have been shown to develop neurological and neurodegenerative phenotypes.

A second group of full-length mHTT rodent models was created using yeast and bacterial artificial chromosome technology (YAC and BAC transgenesis, respectively). Such models express human genomic mutant HTT transgenes along with all the introns and exons, and include regulatory
sequences up to 24-kb upstream and 117-kb downstream of the gene, ensuring appropriate temporal and tissue-specific expression of mHTT\textsuperscript{53-56}. YAC128 HD mice express mutant \textit{HTT} with 128 CAG repeats\textsuperscript{54}, whereas BACHD mice and rats express mutant \textit{HTT} with 97 CAG repeats\textsuperscript{55,56}. The YAC128 and BACHD mice show limited somatic and germ line repeat instability (Vanessa Wheeler, personal communication),\textsuperscript{55} The YAC128 and BACHD rodent models develop progressive motor, cognitive and psychiatric disturbances along with selective striatal and cortical atrophy\textsuperscript{55,57-59}.

Of the full-length mHTT models, the behavioural and neurodegenerative phenotypes tend to be more severe in the transgenic models expressing human mHTT than in the knock-in lines. Although the exact basis of this difference in severity remains unclear, differences in mHTT expression levels or species-specific sequence differences, such as variation in miRNA target sequences (reviewed in REF\textsuperscript{59}), between human mHTT expressed in the transgenic mice and murine mHTT expressed in the knock-in mice may be possible contributors. Furthermore, although the BACHD and YAC128 models share many phenotypic characteristics, there are notable differences between them, including divergent neurochemical and aggregation phenotypes\textsuperscript{60}.

\textbf{Large animal models.} Research using large animal models of HD has been extremely limited. Indeed, before 2008, there was only a handful of papers on the use of non-human primates as models of HD, and there is no literature on the use of cats, dogs or domesticated farm animals as experimental models of HD.

The use of non-human primates before 2008 was largely restricted to the study of HD-like motor dysfunction\textsuperscript{61-63} and the pathogenicity of mHTT\textsuperscript{63} (for other references see REF\textsuperscript{64}), or to the investigation of potential therapies such as neural transplantation\textsuperscript{65} (for other references see REF\textsuperscript{66}) and neurotrophic factor delivery\textsuperscript{67,68}. These studies were conducted in animals with lesions of the basal ganglia caused by quinolinic acid or 3-NPA (for a review of these studies, see REF\textsuperscript{69}). Studies of basal ganglia function demonstrated that non-human primates develop chorea that is qualitatively similar to that seen in human
disease, and that some of the symptoms caused by striatal lesions might be reversed\textsuperscript{9,68,70}. Nevertheless, the limitations of the use of neurochemical rodent models discussed previously apply equally to lesion models of HD in non-human primates. Accordingly, few attempts have been made to study any other aspects of HD using neurochemical monkey models.

The potential use of large animal models in HD research has been refocused recently with the publication of three transgenic large animal models of HD, a non-human primate model (rhesus monkey, \textit{Macaca mulatta})\textsuperscript{71}, a sheep model (\textit{Ovis aries})\textsuperscript{72} and a Tibetan miniature pig model\textsuperscript{73}. The rhesus macaque and pig models were generated using fragments of human \textit{HTT} carrying a CAG repeat expansion. By contrast, the sheep model uses the full-length human coding sequence of \textit{HTT} that is expressed from a transgene. The symptoms exhibited by each species of HD animal reflect the transgene construct used.

The transgenic monkey was made by injecting oocytes with lentiviruses expressing exon 1 of \textit{HTT} carrying 84 repeats\textsuperscript{71}. Expression was driven by the ubiquitin promoter, and no attempt was made to determine the insertion point of the transgene. Five live animals expressing mHTT were carried to term; three carried CAG tracts of 83–88 repeats and showed a severe phenotype, whereas one harboured 29 repeats and was asymptomatic at time of publication (6 months old). The hallmark features of HD, including nuclear inclusions and neuropil aggregates, were observed in the brains of the symptomatic HD monkeys. In fact, the inclusion pathology was extensive, with inclusions in all neurons. The pathology was more similar to that observed in the R6/2 mouse than is typically seen in the human brain. This is probably not surprising, given that the transgene contained, similar to in the R6/2 mouse, an N-terminal HTT fragment and that its expression was controlled by the ubiquitin promoter (ubiquitin is upregulated in HD, so a positive feedback of HTT fragment expression would be expected). Two of the five monkeys died perinatally, whereas two others that survived for at least 1 month showed clinical features of HD, including dystonia and chorea. Again, the early onset of symptoms and unusual symptoms (chorea is not typically associated with juvenile HD) probably
reflects the severity of the model. The extreme phenotype of these animals meant that none, apart from the monkey with the CAG tract of 29 repeats that is non-pathological for humans, survived past 6 months.

The transgenic HD sheep model was made by microinjection of a full-length human HTT cDNA containing 73 CAG repeats under the control of the human HTT promoter. Protein analysis confirmed expression of human mHTT from the transgene and preliminary analysis of one animal at 7 months of age suggested decreased expression of DARPP-32, consistent with the decrease in expression of this striatal marker of medium spiny neurons in brains of patients with HD. It has been reported that there is occasional but characteristic inclusion pathology seen in the brains of HD sheep at 18 months, although no overt phenotype has yet been seen. Again, these results are consistent with the experience from rodent models. Of note, if a mouse line was constructed carrying a full-length HTT transgene carrying 73 CAG repeats, a robust behavioural or neuropathological phenotype would be unlikely to be observed within the lifespan of the mouse (2–3 years).

Nuclear transfer was used to generate transgenic Tibetan miniature pigs that express the N-terminal of mHTT (1–208 amino acids) with a repeat of 105 glutamine residues; expression of the construct was under the control of the cyclomegalovirus enhancer and chicken β-actin promoter. Although five live piglets were born, they failed to thrive, and three died within 3 days of birth. The fourth piglet died after 25 days, leaving a single piglet alive but asymptomatic at 4 months of age. The expression levels of mHTT varied markedly between the piglets, with the surviving piglet showing the lowest level of mHTT expression.

More recently, a lentivirus-based approach has been employed to generate viable transgenic HD (TgHD) minipigs with successful germline transmission. The TgHD minipigs express a truncated N-terminus fragment of mHTT (1–548 amino acids) with 124 glutamines driven by the human HTT promoter in both CNS and non-CNS tissues. In these animals, no aggregate formation in brain tissues is detected up to 16 months of age, and no developmental or gross motor abnormalities are observed. The minipigs exhibit
reduced neostratal DARPP32 levels, as assessed by intensity measurements at 16 months of age, and reduced fertility at 12 months.  

Although the non-human primate, sheep and pig models described above were labelled as the first ‘large’ animal models of HD, in truth, none of them can be considered to be useful models of this disease. Evidence that any of them recapitulates the human disease is scant. Furthermore, the failure of the monkeys to survive means that non-human primate HD models currently do not exist. Only the sheep and TgHD minipig lines have been reproductively viable and capable of transmission of the mutant gene. The absence of a phenotype in the sheep must not be considered as a failure, since a human with a 73 CAG repeat would be unlikely to develop symptoms until early to mid childhood. Nevertheless, until a quantifiable phenotype is described in these sheep, the jury must remain ‘out’ as to how useful they will be as a model of HD.  

A possible confound with all transgenic animal models of HD with random genomic integration of the mutant HTT transgene is the potential for disruption of a functional endogenous gene. The disruption of such genes may indeed influence mHTT-mediated phenotypes.  

Given the limited data available on genetic large animal models of HD, and due to space limitations, in the discussion that follows we provide an overview of the clinical features of HD and focus on the modelling of these features in fruit fly and rodent models of HD.  

**Modelling motor symptoms**  
**Motor dysfunction in Huntington disease.** Disturbances in motor function are the most apparent signs of HD. Chorea, a dance-like involuntary movement, is the major motor symptom in HD and is seen in over 90% of patients with this disorder. Bradykinesia, a slowness in the performance of voluntary movements, and rigidity, a resistance to passive joint movements, often develop as the disease progresses and become dominant in the late stages of the disease. Similarly, dystonia, involuntary muscle contractions that can cause
twisting and abnormal postures, is a feature of advance stages of disease\textsuperscript{79,81}.

Other signs of motor deficits are early impairment of voluntary motor function, difficulties with fine motor control and gait disturbances. Dysarthria, speech abnormalities as a result of dysfunction of the motor component of speech production, is present early in the illness and becomes more pronounced with disease progression. Finally, difficulties in swallowing, termed dysphagia, develop in advance stages of HD and may lead to choking.

**Modelling motor dysfunction in animals.** Tests of motor function in animal models of HD are commonly used to quantify disease progression, and general deficits in motor coordination have been reported in all animal models of HD. Nevertheless, recapitulating the specific features of HD-related motor dysfunction, such as chorea, dysarthria or dysphagia, in the currently available animals models is difficult, at least in part because the animals being studied are either quadrupeds or invertebrates with 6 legs. In *D. melanogaster* models, abnormalities in locomotion have been assessed in larvae and adult flies expressing mHTT (TABLE 1). The crawling behaviour of wandering third-instar larval is reduced in those expressing mHTT\textsuperscript{30}. In adult flies, assessment of motor function relies on the natural tendency of the animals to move against gravity, a climbing behaviour termed ‘negative geotaxis’. Fruit flies expressing mHTT showed a progressive, age-dependent deficit in climbing behaviour compared with those expressing wild-type HTT\textsuperscript{31-33}.

In rodents, a more extensive battery of tests has been used to characterize mHTT-related motor dysfunction, including spontaneous activity measures, the rotarod test, the climbing test, the balance beam test, and footprint gait analysis (for review of motor function tests in rodents, see REF.\textsuperscript{82}). General locomotor activity is measured by monitoring spontaneous or home cage activity using photobeam arrays or video tracking analysis packages.

Overall, locomotion is found to be altered in rodent models of HD, with studies showing locomotor activity deficits in R/1 \textsuperscript{83,84}, R6/2 \textsuperscript{39,85-87}, N171 \textsuperscript{88},
CAG140, YAC128, BACHD mice, and rats although the nature and extent of the deficit depends on test of motor function employed. The rotarod test, arguably the most commonly used test of motor coordination in rodent models of HD, assesses the ability of rodents to maintain balance on a rotating rod. With few exceptions, rodents models of HD, including R6/1, R6/2, N171, YAC128, and BACHD mice and rats, exhibit progressive deficits in rotarod performance. In the climbing test of motor function, mice are placed at the bottom end of a wire mesh cylinder with a sealed top, and the latency to climb (four paws off the ground) and the climbing duration are recorded. Compared with wild-type littermates, the latency to climb, and/or climbing duration has been shown to be decreased in R6/2, YAC128 and BACHD mice. The balance beam test measures the amount of time it takes a mouse to traverse a beam suspended above the ground. R6/1, R6/2, N171, YAC128 and BACHD mice exhibit increased latency to traverse the beams compared with wild-type littermates. In the footprint or gait analysis test, the pattern or manner of the rodent’s movement is measured using an ink or video analysis based approach. Gait abnormalities have been shown in R6/1, R6/2, N171, BACHD mice, and rats, HdhQ111 and CAG140 mice.

Importantly, although many aspects of motor performance can be modelled in mice, other specific involuntary and voluntary motor abnormalities observed in patients with HD, such as involuntary facial movements, oculomotor abnormalities, dysarthria and dysphagia, may be difficult to model, and have as yet not been modelled, in animals.

**Modelling cognitive disturbances**

**Cognitive disturbances in Huntington disease.** A range of cognitive faculties are affected in HD, including executive function, memory, attention and concentration, language-related functions and visuospatial functions. Impairments in cognition can be detected in HD gene carriers many years before clinical diagnosis of disease onset. Although early deficits in cognition are
consistently observed in premanifest HD gene carriers, the impairments are mild at this stage.

In patients with HD, signs of global decline in cognition are pronounced. Executive function deficits are observed, including slowness of thought, changes in personality, and a diminished ability to integrate new information. Memory decline becomes evident in early stage of HD, including impairments in visuospatial memory, information retrieval, and difficulties with recall of recent and remote events\textsuperscript{102,103}. Deficits in procedural memory, associated with difficulties in learning and acquisition of new motor skills for example, are also present. Attention and concentration are also affected, leading to difficulties in organizing and planning, and an inability to initiate and coordinate complex actions. Language-related functions are affected as well and include reduced fluency and loss of conversational initiative in spontaneous speech. Although orientation to time and place are relatively preserved, detailed neuropsychological tests reveal that patients with HD exhibit impaired visuospatial abilities.

\textbf{Modelling cognitive dysfunction in animals.} Although fruit flies have been applied to the study of learning and memory as well as complex behaviours\textsuperscript{104}, to our knowledge no studies of learning and memory in fruit fly models of HD have been reported. By contrast, rodent models have been used extensively to study cognitive deficits in HD. Although the cortical and subcortical (striatal) regions most closely associated with cognitive decline in patients with HD are not as anatomically complex in rodents, many of the critical neuroanatomical and functional characteristics of the CNS are preserved in rodents\textsuperscript{105,106}. This homology has permitted meaningful assessments of cognitive decline in rodents that parallel what is seen in patients with HD.

Deficits in learning and memory have been assessed using a number of sensitive behavioural paradigms, which include tests of procedural learning, spatial learning, associative and non-associative (habituation) learning, discrimination, episodic learning and strategy shifting. Deficits in motor learning
have been demonstrated during the training phases of the running wheel and rotarod tasks. Of note, deficits in motor learning have been shown in CAG140 mice using the running wheel task and in YAC128 and BACHD mice using the rotarod test. Procedural learning deficits have been shown in R6/1, R6/2 and YAC128 mice. These mice have also shown deficits in tasks used to assess spatial learning and memory, including the Morris water maze, Barnes circular maze and Y-maze. Moreover, the same models have shown deficits in discrimination learning in the two-choice swim, the visual cliff avoidance, and non-visual somatosensory-based tests. Through use of classical and operant conditioning, associative learning deficits have been demonstrated in R6/1 and R6/2 mice, and in strategy-shifting tasks, which have been shown to be particularly sensitive to striatal dysfunction, R6/2, YAC128 and BACHD mice exhibit reduced performance compared with wild-type mice. Finally, deficits in the novel objection recognition test of episodic memory have been shown in R6/1, R6/2, HdhQ111 and YAC128 mice.

Although many of the cognitive tests described above aim to assess deficits in corticostratal function, many have also revealed impaired hippocampal function. Indeed, evidence derived from patients with HD and animal models of HD suggest a role for disrupted hippocampal synaptic plasticity and hippocampal dysfunction in this disorder.

Modelling behavioural abnormalities

**Psychiatric and behavioural disturbances in Huntington disease.** Although the onset of HD is clinically diagnosed based on motor performance, symptoms of psychiatric disturbances such as anxiety, irritability, impulsivity, aggression, apathy and depressed mood are prevalent among prediagnostic HD gene carriers and patients with HD. As many as 40–50% of patients with HD are found to experience depression, and depressed mood may precede disease onset by 4–10 years. Moreover, delusions, hallucinations, obsessions and compulsions, and psychosis occur in up to 50% of patients in the advanced
stages of the disease. Finally, suicide is 4- to 6-fold more common in patients with HD than in the general population.

**Modelling psychiatric disturbances in animals.** Attempts to model psychiatric features of HD have only been made in rodents. Depression-like and anxiety-like behaviours have been the most commonly modelled features through use of a number of different tests. To assess depression-like behaviour, the Porsolt forced swim test (FST), tail suspension test (TST), sucrose preference test (SPT) and splash test (ST) have been employed. Depression-like behaviour has been demonstrated in female R6/1 mice using the FST, in N171 mice using the FST, in female HdhQ111 mice using the FST and ST, and in YAC128 and BACHD mice using the FST and SPT. Anxiety-like behaviours have been assessed using the open field test, elevated plus and zero mazes (EPM and EZM, respectively), light–dark choice (LDC), novelty-suppressed feeding test (NSFT) and fear conditioning test. Increases in anxiety-like behaviour have been shown in YAC128 mice in the open field, EZM and LDC test, in BACHD mice in the open field test, EZM, EPM, LDC test, and fear conditioning test, and in CAG140 mice in the LDC and FC tests. Some studies have shown that R6/2 mice exhibit an increase in anxiety-like behaviour in the LDC test, whereas other studies have shown that these mice show a decrease in anxiety-like behavior in the EPM. Similarly, a decrease in anxiety-like behaviour has been shown in the R6/1 mice and BACHD rat model in the EPM. An increase in anxiety-like behaviour was demonstrated in female, but not male, R6/1 and HdhQ111 mice in the NSFT, highlighting sex-specific psychiatric-like phenotypes in these mice. Increased anxiety-like behaviour in male HdhQ111 mice has been shown in the open field test. Other psychiatric disturbances reported in rodent models of HD include deficits in social behaviour and social interactions in R6/1 mice and increased impulsivity in YAC128 mice.

**Modelling other manifestations of Huntington disease**
In addition to the triad of motor, cognitive, and psychiatric symptoms, there are several other symptoms that are emerging to be important in HD, including sleep and circadian disorders\textsuperscript{139-141}. Furthermore, a number of peripheral manifestations characterize HD, including weight loss\textsuperscript{142-146}, skeletal muscle wasting, testicular atrophy\textsuperscript{147} and peripheral immune system alterations\textsuperscript{148,149} (for a detailed review of the peripheral symptoms of HD, see REF.\textsuperscript{150}).

Sleep abnormalities have been reported in patients with HD\textsuperscript{140,151} (for other references, see REF\textsuperscript{141}), but to date only limited information is available about sleep in HD models. To date, sleep abnormalities have been examined in a \textit{D. melanogaster} HD model\textsuperscript{152}, R6/1 mice\textsuperscript{153} and R6/2 mice\textsuperscript{154,155}. Fruit flies expressing mHTT were found to exhibit sleep fragmentation. These sleep perturbations were also seen following siRNA-mediated knockdown of the endogenous huntingtin gene, suggesting a possible role for loss of normal wild-type HTT function in mediating sleep abnormalities\textsuperscript{152}. In R6/1 mice, assessment of spontaneous cortical activity revealed major alterations in the segregation between active and slow-wave sleep states\textsuperscript{153}. Electroencephalogram (EEG) studies showed marked changes in sleep architecture in R6/2 mice compared with wild-type mice\textsuperscript{154,155}. Specifically, quantitative EEGs revealed the emergence of anomalous increases in gamma activity in all stages of sleep and shifts in theta activity during rapid eye movement sleep in the mutant mice. Interestingly, these increases changes in gamma activity were apparent before R6/2 mice developed abnormalities in diurnal physiology or sleep–wake behaviour.

Circadian abnormalities such as disrupted day-night activity patterns have also been reported in patients with HD\textsuperscript{156} and in rodent models including R6/1\textsuperscript{138}, R6/2\textsuperscript{156,157}, CAG140\textsuperscript{157} and BACHD mice\textsuperscript{157}. Body weight alterations have been reported in a number of rodent models of HD: weight loss has been observed in R6/1\textsuperscript{136}, R6/2\textsuperscript{39,86,158}, N171\textsuperscript{40}, HdhQ111\textsuperscript{39}, and CAG140\textsuperscript{159} mice, whereas in YAC128\textsuperscript{60,160} and BACHD\textsuperscript{55,60} mice, increased body weight is observed, which has been shown to reflect overexpression of full-length human HTT\textsuperscript{160,161}.

Other peripheral abnormalities have also been reproduced in some rodent models of HD. Muscle atrophy has been shown in R6/2 mice\textsuperscript{162,163}, which may
reflect increased activation of apoptotic pathways\textsuperscript{164}. Furthermore, testicular atrophy has been shown in R6/2\textsuperscript{165} and YAC128\textsuperscript{147} mice. Finally, aberrant peripheral immune system activation has been demonstrated in R6/2 and YAC128 mice\textsuperscript{148} and impaired macrophage migration has been shown in BACHD mice\textsuperscript{149}.

**Modeling neuropathological features**

*Neuropathology of HD.* The most prominent neuropathological feature induced by mHTT in patients with HD is atrophy of the striatum (caudate nucleus and putamen) with extensive loss of GABAergic medium-sized spiny neurons (MSNs), which make up 90–95\% of striatal neurons\textsuperscript{166}. Atrophy of the cerebral cortex, which is associated with thinning of cortical layers, is also evident\textsuperscript{167} and, together with the severe striatal atrophy, is largely responsible for the reduction in brain weight (by as much as 400 g) that is seen in patients in the advanced stages of HD\textsuperscript{168}. Although the striatal and cortical regions show the most atrophy, the hypothalamus and the hippocampus are also affected\textsuperscript{118,119,169}. Also, in juvenile cases of HD, gross atrophy of the cerebellum may be evident\textsuperscript{170}.

Enkephalin (Enk) and dopamine D2 receptor (D2R)-expressing neurons and substance P and D1 receptor (D1R)-expressing neurons are highly sensitive to mHTT, and indeed comprise the GABAergic MSNs lost in HD\textsuperscript{171}. In contrast to the loss of Enk/D2R-positive and substance P/D1-positive neurons, striatal neuropeptide Y- and somatostatin-positive interneurons are highly resistant to mHTT and relatively spared in HD. Thus, neurochemically there is a loss of Enk, substance P, D1R, and D2R reactivity.

A characteristic feature of HD that is shared with other polyglutamine diseases is the presence of nuclear inclusions and neuropil (or cytoplasmic) aggregates containing mHTT in neuronal (and non-neuronal) tissues of patients with HD\textsuperscript{172-175}.

*Modeling neuropathology in animal models.* Fruit fly models of HD exhibit a progressive degenerative eye phenotype that is associated with mHTT.
expression\textsuperscript{26,28,30,31,33}. In fruit fly models expressing a truncated N-terminal fragment of mHTT, there is a marked nuclear accumulation of mHTT fragments, whereas in flies expressing full-length mHTT, the mutant protein is mostly found in the cytoplasm. Time-dependent formation of mHTT aggregates is seen in flies expressing N-terminal mHTT fragments but not full-length mHTT\textsuperscript{30,33,176}. The lack of visible mHTT aggregates in the full-length mHTT fly model is consistent with findings from full-length mHTT rodent models of HD, in which mHTT aggregates are seen only months after birth.

The neuropathological changes in rodent models of HD, in which the brain structures are more analogous to the primate brain than is the fly nervous system, have been extensively characterized. Atrophy of the striatum has been reported for both N-terminal fragment\textsuperscript{177-179} and full-length mHTT models of HD\textsuperscript{52,54,55,60,180}. In N-terminal fragment models, progressive striatal atrophy is detected as early as 6 weeks of age\textsuperscript{177,178}. In addition to this decrease in striatal volume, atrophy of other structures, such as the hippocampus, and the brain as a whole occurs in parallel\textsuperscript{177,178}. Despite occurring later in the disease process than alterations associated with the N-terminal fragment models, the changes observed in the full-length mHTT model seem to be less generalized, with selective and progressive atrophy of the striatum, globus pallidus, thalamus and cortex\textsuperscript{180}.

The decrease in brain volume in rodent models of HD is generally accompanied by neuronal loss. Striatal neuronal counts are decreased in R6/1\textsuperscript{179} and R6/2 mice\textsuperscript{181} compared with wild-type mice. In addition to the striatum, neuronal loss is also observed in the cerebellum (there is a decrease in the number of purkinje cells) in R6/1 mice\textsuperscript{95}. Furthermore, N171 mice show increased apoptosis of striatal and cortical neurons\textsuperscript{182}. In the full-length mHTT rodent models, striatal and cortical but not cerebellar and hippocampal neuronal loss is observed in YAC128 mice\textsuperscript{54,57,112}. In BACHD mice, an increase in darkly stained degenerating neurons is observed in the striatum, but no loss of striatal neurons is reported\textsuperscript{65}. Decreases in the levels of several striatally enriched transcripts, including those for DARPP-32, Enk, D1R and D2R and cannabinoid
receptor 1, are observed in YAC128 but not BACHD mice\textsuperscript{60}. In the BACHD rats, an age-dependent decrease in striatal D2R binding along with abnormalities of the striosome compartment of the striatum are observed, although no loss of striatal neurons is reported\textsuperscript{56}.

mHTT aggregates and intranuclear inclusions are seen in all rodent models of HD, although with different kinetics and tissue distributions. In the N-terminal fragment models, mHTT aggregates are observed early (on birth)\textsuperscript{181}, whereas in the full-length mHTT models, mutant HTT aggregates appear in an age-dependent manner over time\textsuperscript{56,57,112}.

**Which model is most suited to study HD?**

The question of which species and which particular model to choose for studying HD will obviously depend on the purpose of the research and the particular question of interest. Different species of animals are better suited for modeling certain aspects of HD, and for different applications (see BOX 3).

For example, gait disturbances may be assessed in rodents and in large animals, despite the fact that they are quadrupeds, because many of the motor control systems in mammals overlap. But such a measure would be not be relevant in fruit fly models of HD. By contrast, fruit fly models may be well suited for high-throughput genetic and pharmacological screening approaches, and may be cheaper and quicker to use than rodent or large animal models of HD. Using unbiased and high-throughput approaches can help identify novel pathways and processes that contribute to, or protect against, HD.

Although flies models may be powerful tools to identify cellular processes that are of potential relevance to disease, the limited homology between fruit fly and human proteins means that candidate molecules or pathways identified in flies will still need to be evaluated in mammalian models of HD. Furthermore, because they do not express all the genes present in mammalian genome, the utility of *D. melanogaster* model systems will always be limited, as is illustrated by the following example. Altered proteolysis of mHTT has been implicated in the
pathogenesis of HD, with two classes of proteases, namely caspases and matrix metalloproteinases (MMPs), being involved. Although genetic and pharmacological studies in fruit fly models of HD may be used to examine generally the involvement of these proteolytic families and pathways in HD, assessment of the role of a specific protease or its therapeutic potential in fruit flies may not be feasible. This is because 21 MMPs are expressed in humans, whereas fruit flies express only two MMPs, and although there are at least 14 caspases in humans, fruit flies only express seven caspases.

Rodent models of HD are versatile with established test batteries of motor, cognitive and psychiatric-like features of the disease. The high level of conservation of genes between rodents and humans makes interrogation of specific molecular targets more feasible than it is in flies. In addition to the study of therapeutic potential of identified pathways and targets, the availability of a vast collection of tools for rodent studies allow various approaches to studying the roles of a given protein in HD pathophysiology for example through genetic deletion (knockout) or overexpression (transgenic) approaches, and assessing cell- or tissue-specific effects of mHTT using the flox/Cre system. Moreover, the large number of rodent models of HD, each with its unique set of molecular and phenotypic features, provides an opportunity to exploit differences in their characteristics to address questions of interest and relevance to HD. Expanded CAG repeat tract instability has been observed in somatic and germ cells from patients with HD and may influence disease expression. In the case of rodent models, the CAG repeat tract in R6/2 mice has been shown to be highly unstable, whereas in YAC128 and BACHD mice, the CAG repeat tract, by comparison, is remarkably stable. The availability of these opposite phenotypes thus provides an opportunity to probe for factors that may underlie this difference in repeat tract stability. Furthermore, generally homologous brain regions between the rodent and primate brain allow the study of the regional neuropathological effects of mHTT as well as the effects of mHTT on neural circuitry. However, the relatively limited life-span of rodents (in comparison to the lifespan of humans) places limitations on the ability to study age-dependent
effects. For example, atrophy in rodent models is generally limited unless large CAG repeat tracts are used. Furthermore, given the difference in brain size between rodents and primates, rodent models are not ideal for studies involving medical device-delivered drugs. For similar reasons, the ability to apply imaging modalities in the study of mHTT-induced pathology is constrained by the small brain size of rodents and the limits of resolution of imaging modalities.

However difficult, the push for large animal models should continue. The advantages of pigs and sheep as models of genetic human diseases are that they have large brains, and that they are more similar to humans than they are to mice, in terms of anatomy, lifespan and genetics. In theory, large animals such as sheep and pigs may turn out to be excellent models of HD\textsuperscript{188}. If they are successful in recapitulating the HD pathology, these models will offer a unique opportunity for understanding the relationship between the gene mutation and the neurological decline in HD. Both sheep and pigs offer advantages for biomedical research over non-human primates for economic reasons. Once characterised, they should also provide an acceptable alternative to the non-human primate models of HD that are now under development.

Although the choice of animal model will depend largely on the question of interest, we provide in TABLE 3 an overview of HD disease symptoms and prominent disease mechanisms along with a list of animal models that may be used to study them. This is not meant to be a definitive guide but should serve to highlight the suitability of particular animal models for study of specific research topics.

**Conclusion**

The debilitating effects of HD continue to devastate patients and their families. Although the search for disease-modifying treatments and indeed a cure for this disorder has not yet been successful, the progress that has been made in the past two decades in terms of the development and characterization of HD animal models gives cause for hope. Different animal models of HD offer different advantages, and the choice of the animal model in the continuing research
efforts should ultimately reflect the particular purpose and the question of interest. Despite the challenges involved, the development of large animal models should continue. If successfully developed, these models will offer a unique opportunity for understanding the relationship between the gene mutation and the neurological decline in HD.
Box 1. Assessing the validity of animal models of human disease

The relevance of a given animal model of human disease is often judged on the basis of three broad measures of validity: the animal model’s construct validity, face validity and predictive validity.

**Construct validity**

Construct validity pertains to how closely the animal model reproduces the pathogenic lesion that underlies the disease in humans. For genetic diseases, a model with high construct validity would reproduce the human mutation in the context of the full-length human gene, under the control of the gene’s endogenous promoter. In the context of HD, the construct validity of models expressing full-length human mutant HTT (mHTT) (the genomic sequence or cDNA) is greater than those expressing full-length mouse mutant Hdh. Similarly, models expressing the full-length genomic sequence of mHTT (that is, with exons and introns) have higher construct validity than full-length mHTT cDNA models or N-terminal fragment models (in which exon 1 of mHTT is expressed). Finally, models in which mHTT or a fragment thereof is under the control of the HTT promoter show greater construct validity than models in which the same coding sequence is under the control of a non-HTT promoter.

**Face validity**

Face validity relates to the extent to which the animal model reproduces the symptoms and phenotypes associated with the human disease. For HD, animals with high face validity would reproduce progressive motor and cognitive deficits as well as psychiatric-like disturbances. Moreover, neuroanatomically and histologically, the models would display selective, age-dependent striatal and cortical neuronal loss and atrophy, nuclear inclusions and neuropil aggregates. Other measures that support face validity of an animal model include similarity in peripheral measures, such as immune inflammatory markers, as well as holistic measures of cellular function, including transcriptional, proteomic and metabolic alterations between the animal model and human HD.
Predictive validity

The predictive validity of an animal model is judged based on how closely improvements in response to treatment in the animal model parallel, or predict, improvements in patients. For treatable conditions, the predictive validity of an animal model may be tested, but for diseases such as HD that are without an effective treatment, assessment of the predictive validity of an animal model is currently impossible.
Box 2. Challenges in cloning and manipulating the HTT gene.

HTT is a large gene: it has 67 exons and its genomic sequence (that is, including all exons and introns) is ~170kb. Thus, even the HTT cDNA is very large. The problems associated with cloning and manipulating such a large gene include the difficulty in obtaining an error-free cDNA copy by reverse-transcription from mRNA transcripts (the error rate of Taq polymerase could be as high as 1 mutation per 1 kb of cDNA). Also, the large gene size lowers the likelihood of finding unique and convenient restriction sites that can be used to manipulate the gene. Given the length of the genomic sequence of the gene, it cannot be cloned into a plasmid, in which a maximum of 10-15 kb can be inserted. Instead, genomic-type constructs such as yeast or bacterial artificial chromosomes (YACs or BACs) must be used. Replication of YAC and BAC constructs are inefficient in comparison to plasmid constructs, which results in lower construct DNA yields, making manipulation of YAC/BAC-expressed genes more difficult. Moreover, large genes are more susceptible to shearing in response to mechanical manipulations such as pipetting and injections. As such construct defects are common, and where the complete construct remains intact, the number of constructs that stably integrate into the genome is often low (low copy number), resulting in low expression levels relative to the endogenous levels of expression. Furthermore, the mutation is a variably expanded CAG repeat and repeats of different lengths confer different pathologies, but the CAG repeat is also unstable in vivo. The instability of the CAG repeat mutation means that the CAG repeat length in the HTT transgene of certain animal models may “drift” over generations, leading to phenotypic differences amongst different generations of the same animal model\textsuperscript{39}. Many of these attributes were discovered in the course of developing mouse models of HD.
Box 3. Is it necessary for an animal model to reproduce all the features of the human condition?

The ideal animal model of a disease would reproduce the full spectrum of its features with a time-course that parallels the evolution of these changes in patients. In the case of many neurological disorders, and in particular neurodegenerative diseases, this may not only be an unrealistic goal, but also an impractical one. Indeed, some disease symptoms of HD, such as suicidal ideation and deficits in language-related functions, may be challenging to reproduce in animal models. Neurodegenerative diseases generally develop in adulthood, with age being the most prominent risk factor. Indeed, the average age of onset in HD is around 40 years. Modeling of the most common forms of these illness would entail ageing animal models for periods that extend for many decades, which is not only impossible for most animal models given their short lifespan, but also of limited short-term utility.

As disease features reflect pathological events in common neurobiological substrates, therapies that improve key representative and defining features of the disease may also improve the wider spectrum of disease symptoms, including those difficult to model in animals. At the very least, animal models suitable for reproducing certain features of the disease would be useful in helping shed light on the potential mechanisms underlying the particular disease feature and evaluating candidate therapies targeted for it.

Although the timeframe for disease manifestation in animal models of HD does not reflect the evolution of symptoms in human adult patients, the inverse relationship between the CAG repeat tract length and age of onset has permitted the use of long CAG repeat tracts in animal models to provide an approximation of the pathogenic processes involved in HD under a shortened timeframe.
Figure 1. Genetic attributes of animal models of HD. Animal models of HD differ with respect to a number of genetic design features including a) whether the full-length HD gene had been used or only a portion of it, b) the CAG repeat length, c) whether the HD gene is expressed from a transgene or knocked into the endogenous HD gene locus, and whether the human HD gene was used or the endogenous gene, d) whether HD complementary DNA (cDNA) or genomic DNA complete with introns and regulatory sequences was used, and e) the nature of the promoter used to drive expression of the mutant protein.
Table 1. *Invertebrate* models of HD: *Caenorhabditis elegans* and *Drosophila melanogaster* models

<table>
<thead>
<tr>
<th>Model</th>
<th>Transgene product*</th>
<th>Promoter</th>
<th>polyQ length</th>
<th>Phenotypes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Caenorhabditis elegans</em> Truncated HTT N-terminus fragment models</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Htn-Q95, Htn-Q150</td>
<td>79 and 171 aa of N-terminus fragment (human HTT cDNA)</td>
<td>Osm-10 (sensory neurons: ASH, ASI, PHA, and PHB)</td>
<td>95 150</td>
<td>Age-dependent aggregation, neuronal dysfunction, and neurodegeneration of ASH sensory neurons</td>
<td>Faber et al. 24</td>
</tr>
<tr>
<td>Htt57Q88, Htt57Q128</td>
<td>57 aa of N-terminus fragment (human HTT cDNA) fused to GFP</td>
<td>Pmec-3 (10 neurons including the six touch receptor neurons)</td>
<td>88 128</td>
<td>Mechanosensory defects, morphological abnormalities in neuronal processes, neuronal dysfunction, absence of neurodegeneration</td>
<td>Parker et al. 25</td>
</tr>
<tr>
<td><em>Drosophila Melanogaster</em> Truncated HTT N-terminus fragment models</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HttQ75, HttQ120</td>
<td>132 aa of N-terminus fragment (human HTT cDNA)</td>
<td>gmr</td>
<td>75 120</td>
<td>Degeneration of photoreceptor neurons</td>
<td>Jackson et al. 26, 27</td>
</tr>
<tr>
<td>Httex1pQ93</td>
<td>65 aa of N-terminus Htt fragment</td>
<td>elav (pan neuronal) gmr (all compound eye cells)</td>
<td>93 97</td>
<td>Progressive loss of rhabdomeres (photoreceptor degeneration) 70% lethality of larvae Reduced survival time in adults</td>
<td>Steffan et al. 26, 29</td>
</tr>
<tr>
<td>Htt128Q</td>
<td>548 aa of N-terminus fragment (human HTT cDNA)</td>
<td>elav (pan neuronal) gmr (all compound eye cells)</td>
<td>128</td>
<td>Reduced locomotion speed in lavae Abnormal motor behaviour in adults Reduced survival time in adults Photoreceptor degeneration</td>
<td>Lee et al. 30</td>
</tr>
<tr>
<td>N-termHttQ128</td>
<td>208 aa of N-terminus fragment (human HTT cDNA)</td>
<td>elav (pan neuronal) gmr (all compound eye cells)</td>
<td>128</td>
<td>Progressive deficits in climbing behavior Reduced survival Neurodegenerative phenotype in the eye</td>
<td>Kaltenbach et al. 31, Miller et al. 32</td>
</tr>
<tr>
<td><em>Drosophila Melanogaster</em> Full-length HTT models</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Htt128QFL</td>
<td>Full-length HTT cDNA</td>
<td>elav (pan neuronal) gmr apVNC (large CNS neurons)</td>
<td>128</td>
<td>Progressive degenerative phenotype in eyes Progressive impairment in climbing behavior Electrophysiological abnormalities (increased neurotransmitter efficiency; calcium homeostasis)</td>
<td>Romero et al. 33</td>
</tr>
</tbody>
</table>

* Excluding the polyglutamine tract
Table 2. Mouse models of HD

<table>
<thead>
<tr>
<th>Model</th>
<th>Transgene product</th>
<th>Promoter</th>
<th>CAG repeat length</th>
<th>mHTT expression (relative to endogenous levels)</th>
<th>Reference:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Truncated N-terminus fragment models</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R6/1 mice</td>
<td>67 aa N-terminus fragment (human HTT)</td>
<td>1 kb human HTT promoter</td>
<td>116</td>
<td>~0.30x</td>
<td>Mangiarini et al. 35</td>
</tr>
<tr>
<td>R6/2 mice</td>
<td>67 aa N-terminus fragment (human HTT)</td>
<td>1 kb human HTT promoter</td>
<td>144*</td>
<td>~0.75x</td>
<td>Mangiarini et al. 35</td>
</tr>
<tr>
<td>N171 mice</td>
<td>171 aa N-terminus fragment (human HTT cDNA)</td>
<td>Mouse PrP promoter</td>
<td>82</td>
<td>~0.10-0.20x</td>
<td>Schilling et al. 40</td>
</tr>
<tr>
<td>HD94</td>
<td>67 aa N-terminus fragment (chimeric human:mouse exon 1)</td>
<td>CamKIIα</td>
<td>94</td>
<td>Not reported</td>
<td>Yamamoto et al. 189</td>
</tr>
<tr>
<td>Shortstop mice</td>
<td>171 aa N-terminus fragment (human HTT)</td>
<td>Human HTT promoter and regulatory elements (24kb upstream)</td>
<td>128</td>
<td>~1.00x</td>
<td>Slow et al. 190</td>
</tr>
<tr>
<td>N118-82Q mice</td>
<td>118 aa N-terminus fragment (human HTT cDNA)</td>
<td>Mouse PrP promoter</td>
<td>82</td>
<td>Not reported</td>
<td>Tebbenkamp et al. 191</td>
</tr>
<tr>
<td>N586-82Q mice</td>
<td>586 aa N-terminus fragment (human HTT cDNA)</td>
<td>Mouse PrP promoter</td>
<td>82</td>
<td>&gt;1.0x</td>
<td>Tebbenkamp et al. 192</td>
</tr>
<tr>
<td>Ubi-G-HTT84Q mice</td>
<td>67 aa N-terminus fragment (human HTT) fused to EGFP</td>
<td>Ubiquitin promoter</td>
<td>84</td>
<td>Not reported</td>
<td>Cheng et al. 193</td>
</tr>
<tr>
<td>HD150QQ, HD190QQ</td>
<td>67 aa N-terminus fragment (human HTT) fused to EGFP</td>
<td>1 kb human HTT promoter</td>
<td>150, 190</td>
<td>~10.00x, ~2.50x</td>
<td>Kotliarova et al. 194</td>
</tr>
<tr>
<td>Model Type</td>
<td>Breed</td>
<td>Details</td>
<td>Promoter Region</td>
<td>Expression Level</td>
<td>References</td>
</tr>
<tr>
<td>----------------------------</td>
<td>----------------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>--------------------------------------</td>
<td>---------------------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td><strong>Full-length HD models:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knock-in models</td>
<td>CAG140 mice</td>
<td>Full-length chimeric human HTT exon 1:mouse Htt</td>
<td>Endogenous mouse Hdh promoter</td>
<td>119, 140</td>
<td>Expressed at endogenous levels</td>
</tr>
<tr>
<td></td>
<td>HdhQ92 mice</td>
<td>Full-length chimeric human HTT exon 1:mouse Htt</td>
<td>Endogenous mouse Hdh promoter</td>
<td>92</td>
<td>Expressed at endogenous levels</td>
</tr>
<tr>
<td></td>
<td>HdhQ111 mice</td>
<td>Full-length chimeric human HTT exon 1:mouse Htt</td>
<td>Endogenous mouse Hdh promoter</td>
<td>111</td>
<td>Expressed at endogenous levels</td>
</tr>
<tr>
<td></td>
<td>HdhQ150, HdhQ200 mice</td>
<td>Full-length chimeric human HTT exon 1:mouse Htt</td>
<td>Endogenous mouse Hdh promoter</td>
<td>150, 200</td>
<td>Expressed at endogenous levels</td>
</tr>
<tr>
<td></td>
<td>Hdh4/Q72 and Hdh4/Q80 mice</td>
<td>Full-length chimeric human HTT exon 1:mouse Htt</td>
<td>Endogenous mouse Hdh promoter</td>
<td>72, 80</td>
<td>Expressed at endogenous levels</td>
</tr>
<tr>
<td></td>
<td>zQ175 mice</td>
<td>Full-length chimeric human HTT exon 1:mouse Htt</td>
<td>Endogenous mouse Hdh promoter</td>
<td>188</td>
<td>Expressed at endogenous levels</td>
</tr>
<tr>
<td><strong>Transgenic models</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD48 and HD89 mice**</td>
<td>YAC128 mice</td>
<td>Full-length human HTT</td>
<td>Human HTT promoter and regulatory elements (24kb upstream, 117kb downstream)</td>
<td>128</td>
<td>~1.00x</td>
</tr>
<tr>
<td></td>
<td>YAC48 mice</td>
<td>Full-length human HTT</td>
<td>Human HTT promoter and regulatory elements</td>
<td>48</td>
<td>~0.3-0.5x</td>
</tr>
<tr>
<td></td>
<td>YAC72 mice</td>
<td>Full-length human HTT</td>
<td>Human HTT promoter and regulatory elements</td>
<td>77</td>
<td>~0.3-0.5x (line 2511); ~2.0x (line</td>
</tr>
<tr>
<td>Model</td>
<td>Full-length HTT or Htt</td>
<td>Human HTT or Htt cDNA</td>
<td>Promoter and regulatory elements</td>
<td>Expansion Factor</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------</td>
<td>------------------------</td>
<td>-----------------------</td>
<td>----------------------------------</td>
<td>------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>BACHD mice</td>
<td>Full-length human HTT</td>
<td>Human HTT promoter and regulatory elements (20kb upstream, 50kb downstream)</td>
<td>97</td>
<td>~1.36x</td>
<td>Gray et al.</td>
</tr>
<tr>
<td>Hu97/18 mice</td>
<td>Fully humanized mouse model: two full-length human HTT genes, no mouse Hdh genes</td>
<td>Human HTT promoter and regulatory elements</td>
<td>97</td>
<td>~1.0x***</td>
<td>Southwell et al.</td>
</tr>
<tr>
<td>iFL148Q mice</td>
<td>Full-length Htt (inducible)</td>
<td>Htt cDNA</td>
<td>148</td>
<td>~1.0x</td>
<td>Tanaka et al.</td>
</tr>
<tr>
<td>BACHD rats</td>
<td>Full-length human HTT</td>
<td>Human HTT promoter and regulatory elements</td>
<td>97</td>
<td>~2.5-4.5x</td>
<td>Yu-Taeger et al.</td>
</tr>
</tbody>
</table>

* unstable with expansions > 250 CAG repeats reported; ** these mice have been lost and are no longer available; *** relative to full-length human HTT-18Q transgene
Table 3. Huntington disease features, mechanisms and study approaches, and models most suited for them

<table>
<thead>
<tr>
<th>Huntington disease symptom, mechanism, study approach</th>
<th>Drosophila</th>
<th>Rodents</th>
<th>Pigs/sheep</th>
<th>NHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>N FL</td>
<td>N FL</td>
<td>N KI</td>
<td>hFL</td>
<td></td>
</tr>
</tbody>
</table>

**Experimental approaches**

- Large-scale screening of genetic and/or pharmacological modifiers of disease phenotype
  - N
  - FL
  - Rodents
  - Pigs/sheep
  - NHP

- Medical device-based CNS drug-delivery
  - N
  - FL
  - Rodents
  - Pigs/sheep
  - NHP

- Conditional and/or tissue-specific expression
  - N
  - FL
  - Rodents
  - Pigs/sheep
  - NHP

- Genetic crosses to investigate the role of specific genes in HD
  - N
  - FL
  - Rodents
  - Pigs/sheep
  - NHP

**Disease mechanisms**

- Study of the role of full-length protein context in HD
  - N
  - FL
  - Rodents
  - Pigs/sheep
  - NHP

- Study of the role of human HTT genomic context in HD
  - N
  - FL
  - Rodents
  - Pigs/sheep
  - NHP

**Disease symptoms and features**

- Motor dysfunction
  - N
  - FL
  - Rodents
  - Pigs/sheep
  - NHP

- Cognitive deficits
  - N
  - FL
  - Rodents
  - Pigs/sheep
  - NHP

- Psychiatric-like disturbances
  - N
  - FL
  - Rodents
  - Pigs/sheep
  - NHP

- Disrupted circadian rhythm
  - N
  - FL
  - Rodents
  - Pigs/sheep
  - NHP

- Sleep disturbances
  - N
  - FL
  - Rodents
  - Pigs/sheep
  - NHP

- Metabolic abnormalities (changes in weight, muscle mass)
  - N
  - FL
  - Rodents
  - Pigs/sheep
  - NHP

N = N-terminus HTT fragment models; KI = knock-in models; hFL = full-length human HTT models; NHP = non-human primate
× not feasible, or impractical; ● feasible and has been examined; ○ is feasible but has not been examine/reported
Competing interests statement
The authors declare no competing financial interests.

References


60. Pouladi, M. A. et al. Marked differences in neurochemistry and aggregates despite similar behavioural and neuropathological features of


110. Brooks, S. P., Jones, L. & Dunnett, S. B. Longitudinal analyses of operant


Asymptomatic sleep abnormalities are a common early feature in patients with Huntington's disease. 


Tebbenkamp, A. T. N., Swing, D., Tessarollo, L. & Borchelt, D. R.


Glossary

Yeast or bacterial artificial chromosome (YAC or BAC). DNA vectors engineered to replicate in yeast (YAC) or bacteria (BAC) and used to clone very large pieces of DNA.

UAS/GAL System. A bipartite enhancer system widely used in fruit fly studies that allows targeted expression of genes of interest in specific tissues.

Knock-in model. A genetic animal model in which the alteration comprises insertion of extragenomic DNA at a specific locus in the animal’s genome.

Chorea. An irregular, jerky, dance-like involuntary movement that is characteristic of a number of movement disorders, including Huntington disease.

Dystonia. Involuntary muscle contractions that can cause twisting and abnormal postures.

Bradykinesia. A slowness in the performance of voluntary movements.

Dysarthria. Speech abnormalities as a result of dysfunction of the motor component of speech production.

Dysphagia. A difficulty in swallowing.
Highlighted References


First reports implicating aberrant glutamate signaling in HD, and spurring the development of the earliest non-genetic (lesional) animal models of HD.


First evidence of aberrant glutamate signaling and enhanced susceptibility to NMDAR-mediated toxicity in a transgenic animal model of HD.


Demonstrates that transgenic expression of a mHTT N-terminus fragment in C. elegans, which lack a HTT ortholog, is sufficient to cause age-dependent neuronal dysfunction and, on a sensitized background, degeneration.


First reported fruit fly model of HD.


This study reports on the first transgenic mHTT N-terminus fragment rodent model of HD.

41. White, J. K. et al. Huntingtin is required for neurogenesis and is not impaired by the Huntington's disease CAG expansion. Nat Genet 17, 404–
First knock-in rodent model of HD.


Reports on the generation of the first full-length human mHTT rodent model of HD using YAC transgenesis.


Characterization of the first full-length human mHTT rat model of HD.


Along with Pang et al. (ref 131), using a transgenic rodent model, the study points to a neurobiological basis for depressive symptoms in HD.


Describes the first attempt to establish a transgenic non-human primate model of HD. Demonstrates that transgenic overexpression of a mHTT N-terminus fragment results in severe neurological phenotypes.


Reports on the generation of a full-length HTT cDNA sheep model of HD.


Describes the first attempt to establish a transgenic minipig model of HD.

First transgenic truncated N-terminus mHTT fragment minipig model of HD with successful germline transmission.


Along with Pouladi et al. (ref 58), using a transgenic rodent model, the study points to a neurobiological basis for depressive symptoms in HD.


Characterization of the first truncated N-terminus mHTT fragment rat model of HD.
Online Summary

- Animal models of HD, established in species that range from worms, fruit flies, mice, and rats to pigs, sheep, and monkeys, have provided important insights into the pathogenesis of this disease.
- Key distinguishing factors among animal models of HD are the genetic approach with which they were generated and the nature of the \textit{HTT} mutation that they carry.
- The symptoms exhibited by each model largely reflect the genetic approach and transgene construct used to generate them.
- Rodents are by far the most commonly used animals for modeling HD, with over 20 different models having been generated.
- Different species of animals are better suited for modeling certain aspects of HD, and for different applications. The choice of species and the particular model to use will therefore depend on the specific question of interest.
- The goal of generating large animal models of HD should be pursued as certain challenges with regard to developing therapeutics for HD cannot be met in rodents and other small animals.
Author biographies

Mahmoud A. Pouladi is an Assistant Professor at the National University of Singapore, and an Assistant Principal Investigator at the Agency for Science, Technology and Research (A*STAR), Singapore. He received his Masters degree from McMaster University (Hamilton, Ontario, Canada), and a PhD in Medical Genetics from the University of British Columbia (Vancouver, BC, Canada). His research focuses on understanding the neurobiology of neurodegenerative diseases, in particular Huntington disease, and to develop novel approaches for therapeutic intervention.

Jenny Morton is Professor of Neurobiology at the University of Cambridge, United Kingdom. She has a long-standing interest in the pathophysiology of HD, particularly at the earliest stages of disease, where she thinks therapies are likely to have the greatest impact. Her research has concentrated on cognitive dysfunction, sleep disturbance and circadian abnormalities in HD mouse models. More recently, the focus of her laboratory has shifted to studying behaviour in large animal models, in particular the HD sheep.

Michael R. Hayden is a Senior Scientist at the Centre for Molecular Medicine and Therapeutics, Killam University Professor at the University of British Columbia (Vancouver, BC, Canada), and holds a Canada Research Chair in Human Genetics. Dr. Hayden is also President of Global R&D & Chief Scientific Officer at Teva Pharmaceuticals (Petah Tikva, Israel), and Programme Director and Distinguished Professor at the Translational Laboratory in Genetic Medicine, A*STAR, and the National University of Singapore (Singapore). His work focuses on understanding the genetic roots of illness and using that understanding to develop better approaches to treatment for patients. Much of his career has been dedicated to understanding the development of Huntington disease and finding a way to cure it. He cofounded the Canadian National Program in Drug Safety (CNPDS) which is focussed on detection and determination of the genetic causes for adverse drug reactions.