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Mouse Models of Huntington's Disease and Methodological Considerations for Therapeutic Trials

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Abstract

Huntington's disease (HD) is an autosomal dominant, progressive, and fatal neurodegenerative disorder caused by an expanded polyglutamine cytosine-adenine-guanine repeat in the gene coding for the protein huntingtin. Despite great progress, a direct causative pathway from the HD gene mutation to neuronal dysfunction and death has not yet been established. One important advance in understanding the pathogenic mechanisms of this disease has been the development of multiple murine models that replicate many of the clinical, neuropathological, and molecular events in HD patients. These models have played an important role in providing accurate and experimentally accessible systems to study multiple aspects of disease pathogenesis and to test potential therapeutic treatment strategies. Understanding how disease processes interrelate has become important in identifying a pharmacotherapy in HD and in the design of clinical trials. A review of the current state of HD mouse models and their successes in elucidating disease pathogenesis are discussed. There is no clinically proven treatment for HD that can halt or ameliorate the inexorable disease progression. As such, a guide to assessing studies in mouse models and salient issues related to translation from mice to humans are included.

Keywords

Huntington's disease; polyglutamine repeat; toxin models; genetic models; translational studies

Introduction

Huntington's disease (HD) is an autosomal dominant inherited neurodegenerative disorder that is characterized by progressive motor dysfunction, emotional disturbances, dementia, and weight loss. Although the disorder had previously been reported, the initial detailed description of HD was that of George Huntington, a medical practitioner of Pomeroy, Ohio, in 1872 [1]. He gave a detailed account of the disease based upon the descriptions taken by his father and grandfather from their practice in East Hampton, Long Island, NY. Those patients could be traced to a few individuals that had emigrated from a small village in Suffolk, England in 1630. Neuropathologists had previously described HD as a chronic encephalitis [2]. In 1908, however, Jergelsma first described the characteristic neuropathologic alterations within the basal ganglia [3]. HD occurs worldwide, in all races and ethnic groups [4]. Its prevalence is 5-10 cases per 100,000, and there is a new mutation rate as high as 1-3% [5]. There are about 30,000 affected individuals in the United States. Another 150,000 Americans have a genetic risk for developing the disease. The average age of onset is 38 years of age, however the range is from infancy into the ninth decade. There is increasing reason to believe that pathologic

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alterations occur in the brain years before symptoms manifest [6,7,8]. A recent MRI study of gray matter in presymptomatic HD patients close to estimated diagnostic onset, using a weighted VBM voxel procedure, showed degenerative changes in the striatum, in contrast to control subjects [8]. Once symptomatic, affected individuals are rapidly disabled by early functional decline, and require increasing care and supervision for another 15-25 years before succumbing to the effects of severe physical and mental deterioration. In approximately twenty-five percent of patients with HD, the first symptoms appear after age 50 [9]. The rare juvenile form of HD, which is associated with the rigid-type or Westphal variant, is found in 2% of the total population having the disease [10]. Because of early functional decline, the chronic and the increasingly intensive multidisciplinary care it requires, and its genetic nature, HD disproportionately consumes medical, social, and family resources [11].

The HD gene, first cloned in 1993 [12], codes for a large, highly conserved protein (huntingtin) of unknown function that is found in neurons throughout the brain. Emerging evidence, however, suggests it is involved in fast axonal transport [13,14], specifically enhancing vesicular transport of brain-derived neurotrophic factor along microtubules [15]. Rather than a simple missense mutation or deletion, the HD gene defect is an expanded unstable DNA segment, containing a polymorphic trinucleotide cytosine-adenine-guanine (CAG) repeat in the coding sequence of the IT15 gene on chromosome 4. In individuals with HD, a polymorphic trinucleotide repeat sequence (CAGn), near the 5' end of the gene, is expanded beyond the normal repeat range, leading to the translation of an expanded polyglutamine sequence in the protein. In the normal population, the number of CAG repeats varies from 17 to 29. In individuals with HD, there are more than 37 repeats. Once expanded into the pathogenic ranges, there is an inverse relationship between the CAG repeat number and the age of onset, with higher repeat associated with younger age and greater severity. In HD, both normal and mutant alleles are expressed. Gain-of-function alterations in which mutant huntingtin has protein-level toxicity, as well as loss of function of normal huntingtin, have been proposed to contribute to HD.

Expansion of trinucleotide repeats is now well recognized as a major cause of neurological disease [16-18]. HD is one member of the family of neurodegenerative triplet repeat disorders with anticipation and a gain- or change-of-function mutation. These include spinocerebellar ataxias, dentato-rubro-pallido-luysion atrophy, Machado-Joseph disease, and spinal bulbar muscular atrophy. As in HD, selective loss of neurons underlies these diseases and misfolding and abnormal aggregation of the mutant protein occur. It has thus been hypothesized that neurodegeneration in these disorders may have similar molecular bases. Interestingly, the pathogenic mechanisms in HD are also themes relevant for other neurodegenerative disorders, including Parkinson's, amyotrophic lateral sclerosis, and Alzheimer's diseases.

Despite great progress, a direct causative pathway from the HD gene mutation to neuronal dysfunction and death has not yet been established. Although the exact cause of neuronal death in HD remains unknown, it has been postulated that proteolysis of mutant huntingtin plays a role in disease pathogenesis, resulting in an abnormal and toxic amino acid (N)-terminal fragment that forms protein aggregates in neurons. While these aggregates induce chaperone expression and become ubiquitinated, they persist, indicating protein misfolding [19] and failed proteolysis [20]. They also sequester a variety of other important cellular proteins including chaperones [21], proteasomal proteins [22], normal huntingtin [19], and transcription factors [23], as another means of disturbing protein homeostasis [24]. These aggregates can be readily detected throughout the course of the disease [25-27]. While no one specific protein-protein interaction of mutant huntingtin has been suggested to be the pathologic trigger, evidence has accumulated that direct interactions between mutant huntingtin and transcription factors may be the proximal defect that underlies transcriptional dysfunction [23]. This then results in a cascade of compensatory and damaging events to molecular processes and genetic programs

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that ultimately lead to neuronal death. These pathological events include oxidative stress, mitochondrial dysfunction, altered receptor activities, inflammation, pro-apoptotic signals, bioenergetic defects, increased transglutaminase activity, and excitotoxicity [28].

Basal ganglia pathology has been the most thoroughly characterized and has been central to the development of animal models and hypotheses about the circuitry involved in chorea and about potential mechanisms of neuronal death in HD. While cerebral cortical changes in HD occur early and are topographically selective to subcortical white matter in premanifest disease [29,30], the most striking early neuropathological feature is marked gross atrophy of the neostriatum with concomitant neuronal degeneration within the caudate nucleus and putamen [31,8]. There is a topographic progression of neuronal loss and astrogliosis first observed in the dorso-medial aspect of the striatum and progressing ventro-laterally, with relative sparing of the ventral striatum [32]. The striosomal/matrix pattern in the neostriatum is topographically altered in HD, with the total area of matrix reduced and the area of the striosomes unchanged. These findings are consistent with the preferential loss of striatal D1 receptors. Not all neurons, however, are affected equally within the neostriatum [33]. The hallmark of neuropathology in HD is selective neurodegeneration in which vulnerable populations of neurons degenerate, while less vulnerable populations are spared [33]. While large cholinergic neurons and medium-sized nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase aspiny neurons remain relatively spared in HD, medium-sized spiny γ-aminobutyric acid (GABA)-ergic projection neurons of the striatum, which make up roughly 90% of striatal neuron content, are disproportionately affected early and most severely [34-36]. There is also a reduction in striatal neurochemicals that parallels striatal neurodegeneration in HD [37], with enkephalin-expressing striatal projection neurons appearing more vulnerable in early adult-onset HD, in comparison to substance P striatal neurons [38]. It is of interest to note that substance P is found in both spiny and aspiny neurons in a 70:30 % ratio respectively [39]. It is unclear is depleted in HD. Some substance P neurons, however, are still present throughout HD striatum. The majority of these preserved neurons have been characterized as aspiny, using light and electron microscopic methods [40]. In addition, striatal medium spiny neurons show both proliferative and degenerative changes in the prolonged process leading to neuronal dysfunction and cell death [41,42].

The pathogenic significance of cytosolic and nuclear mutant huntingtin aggregation remains unclear. Ongoing debate continues, as with other neurodegenerative disorders in which protein aggregates are a hallmark of disease, questioning whether inclusions, otherwise referred to as intracellular agglomerates, formed by the aggregated N-terminal truncation of huntingtin cause neuronal death through alterations of nuclear transport or DNA arrangements affecting transcription [23]. Nuclear inclusions are largely made up of cleaved N-terminal products, whereas cytoplasmic inclusions contain both cleaved and larger intact proteins [43]. In general, huntingtin aggregates are ubiquitous and in large numbers throughout HD mouse brain models [44], while largely confined to the neocortex with little or no aggregates observed in the neostriatum of HD patients [25,27]. It is unlikely that the latter can be explained by unrecognized aggregates or their rapid formation and loss. Nuclear huntingtin and ubiquitin aggregation is present well before the neurological deficits occur in transgene animal models, implicating nuclear aggregation in neuronal dysfunction and subsequent death in HD [45,46]. Interestingly, ‘shortstop’ YAC transgenic mice have widespread nuclear huntingtin inclusions, yet demonstrate no significant HD phenotype [47] and suggest that not all mutant huntingtin fragments are toxic [48]. As such, rather than a harbinger of neuronal death, mutant huntingtin aggregation may be a cytoprotective mechanism inactivating polyglutamine-induced neurotoxicity by sequestering the mutant protein until ubiquitination can dismantle it. As such, huntingtin inclusions may not be a key player in the pathogenic events leading to selective neuronal death nor a predictor of neuronal death [27,49-51]. It may well be that soluble mutant huntingtin fragments engage pathologic interactions and that neuronal death may be dependent

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upon the levels of diffuse unaggregated huntingtin found in affected neurons and provide an explanation for the pattern of striatal neuron loss [52,53]. It is difficult, however, to reason that such factors as the mass effect of cytosolic and nuclear huntingtin aggregate burden, the sequestration of essential cellular transcription factors and neuronal proteins and their subsequent reduced activity [54], altered proteosomal function [55], and the localization of mutant huntingtin aggregates to cellular organelles such as mitochondria [56], do not have a deleterious effect upon neuronal function and survival. In defense of this comment, select therapies reduce huntingtin aggregates in HD mouse models and are thought to be responsible, at least in part, for the significant improvement of the behavioral and neuropathological phenotype found in these mice [28,57]. In addition, the blockade of mutant huntingtin in a conditional murine model of HD reduces inclusions and ameliorates the behavioral phenotype [58].

While great strides have been made in understanding the pathogenesis of HD, there is still an incomplete understanding of the disease mechanisms. Animal toxin and genetic models that closely mimic the neurobiological and clinical symptoms of the disease may provide an alternative approach for the study of HD molecular pathogenesis and in the development of existing treatments and novel therapeutic strategies for HD. Therefore, animal models are a crucial part of this rapidly advancing field of HD research. In this chapter, animal models are reviewed in association with implications in uncovering pathogenic mechanisms. In addition, the development of therapies is discussed along with a guide to assessing preclinical trials in mouse models.

Toxin Models of HD

Excitotoxin Lesions in Animals

A suitable animal model of HD must at least replicate the neuropathological features of the disorder as described above. An increasing body of evidence suggests that excitotoxicity plays a role in both acute and chronic neurological diseases. One of the earliest experimental models of HD was established in rats and mice and included the direct introduction of excitatory agonists into the central nervous system. Lucas and Newhouse first observed retinal degeneration in mice after systemic administration of glutamate [59]. This was followed by the demonstration that excitatory amino acids mediated cell death in anoxic hippocampal cultures [60]. The initial observation suggesting that excitotoxicity may play a role in HD was made by the McGeers and Coyle and Schwarcz [61,62]. Both groups of investigators showed that injections of the excitatory glutamate-type neurotoxin, kainic acid, produced degeneration of striatal GABAergic projection neurons with preservation of striatal afferents, resembling the neuropathological sequelae observed in HD. This model was refined by using N-methyl-D-aspartate (NMDA) type excitotoxins, including quinolinic acid, an endogenous intermediate in the kynurenine pathway of tryptophan metabolism, produced a more accurate model of HD. Quinolinic acid is elevated in HD patients, the increased levels possibly derived from activated microglia, resulting in neurodegeneration [63]. Unlike kainic acid, quinolinic acid shows differential sparing of striatal neurons. Quinolinic acid damaged both GABAergic and substance P-containing neurons, with relative sparing of NADPH-diaphorase and cholinergic neurons, that latter of which are known to be spared in HD [34,35]. Of interest to note, quinolinic acid administration results in an age-dependent decrease in enkephalin neuron vulnerability in contrast to substance-P striatal neurons [64]. Others and we have demonstrated that intrastriatal injection of quinolinic acid produced a more accurate model of HD [65,66]. Other NMDA agonists also reproduce relative sparing of NADPH-diaphorase neurons [67]. Chronic quinolinic acid lesions, where months have passed to allow resorption of the necrotic injection site, closely reproduce the patterns of selective neuronal sparing in the rat striatum and cerebral cortex found in HD [68]. Excitotoxin lesions in the monkey, using quinolinic acid, provide an experimental primate model that closely resembles the neuropathological,

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neurochemical, and clinical features of HD [66]. These lesions were characterized by a central zone of intense astrogliosis and marked neuronal depletion. Immunocytochemical and enzyme histochemical markers for both large and medium-sized aspiny striatal neurons and spiny striatal neurons clearly demonstrated a selective pattern of neuronal vulnerability and relative resistance to the excitotoxic effects of quinolinic acid within lesioned striata. There was a disproportionate involvement of the matrix compartment similar to that seen in HD. These changes were accompanied by behavioral alterations suggestive of a hyperkinetic movement disorder. Dopaminergic agonist-inducible chorea was observed in the primates and was indistinguishable from that seen in HD [69]. Ultrastructural analysis confirmed axon-sparing lesions with neuronal loss and astrogliosis. Nonphosphorylated neurofilament immunoreactivity was reduced in cell bodies but increased in axons, consistent with NMDA-mediated activation of the calcium-dependent phosphatase, calcineurin, which dephosphorylates neurofilament protein [70]. These animal models demonstrate a characteristic profile consistent with the features of HD and strongly suggest that an excitotoxic process plays a role in the pathogenesis of this disorder. If an NMDA excitotoxic process plays a role in the selective neuronal degeneration in HD, neurons containing high densities of these receptors should be preferentially vulnerable, resulting in a depletion of these receptors. This is indeed the case. Young and colleagues have shown that NMDA receptors are differentially depleted as compared to other receptor subtypes [71].

It is well established that striatal excitotoxic lesions depend on corticostriatal glutamatergic inputs [72,73]. There is also a substantial body of evidence indicating that striatal excitotoxic lesions are dependent on dopaminergic inputs from the substantia nigra [72-74]. Nigrostriatal dopaminergic neurotransmission is altered in HD based on early and dramatic changes in related receptors and may contribute to striatal vulnerability in HD. More directly, released dopamine can be a stressor to striatal neurons through oxidative mechanisms, as well as modulation of glutamate release [75-77]. It is possible that both cortical and nigral afferent inputs may be responsible for the regional selectivity of neuronal degeneration that is observed in HD.

Defective Energy Metabolism Toxin Models

While an abnormality affecting the NMDA receptor or an increased endogenous excitotoxin could be responsible for HD, candidate toxins, such as quinolinic acid, however, are not increased in this disease. A novel hypothesis explaining the pattern of degeneration in HD has evolved based on animal studies and observations in HD patients. It suggests that impaired cellular energy may be involved in the degenerative process [78,79]. The initial relevant observations were made by Olney who showed that partial membrane depolarization can produce NMDA receptor mediated excitotoxicity by removing the voltage-dependent magnesium block of the NMDA-linked calcium channel [80-81]. The open calcium channel permits normal amounts of endogenous glutamate to produce NMDA receptor-mediated neurotoxicity. Evidence suggests that energy depletion producing partial membrane depolarization also produces NMDA-type excitotoxic lesions [81-82].

It is of great interest that energy failure may be the consequence of impaired electron transport chain function, with subsequent reduced ATP stores that result in membrane depolarization, removal of magnesium from the NMDA-linked calcium channel, and subsequent excitotoxic injury. The electron transport chain is comprised of a series of enzymes, consisting of five major complexes. It is one of a series of reactions that are involved in oxidative phosphorylation. A proton gradient generated by the electron transport chain complexes I, II, and III, stores potential energy for the synthesis of ATP along with reducing oxygen to water. Impaired electron transport chain function produces energy failure that results in membrane depolarization, removal of magnesium from the NMDA-linked calcium channel, and

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subsequent excitotoxic injury. Several electron transport chain enzymes have been shown to be altered in HD. Cytochrome oxidase (Complex IV) abnormalities have been reported in HD caudate nucleus [83]. The loss of cytochrome oxidase in damaged brain areas, however, may be a consequence of neuronal loss rather than a cause. Studies of platelets from HD patients suggest that Complex I activity may also be selectively decreased in HD patients, although it is normal in at-risk family members [84]. Other electron transport chain complexes, including Complex II (succinate ubiquinol oxidoreductase), Complex III (ubiquinol cytochrome c reductase) and Complex IV (cytochrome oxidase) are normal in blood platelets [84]. Decreased activity of Complex II/III of the electron transport chain has been found in the caudate nucleus, but not in other brain areas in HD [85]. Beal and colleagues have demonstrated a 22% decrease in Complex II-III activity in the caudate nucleus and a significant increase in Complex I activity in the frontal cortex, which may be compensatory [86]. It is possible that the mutation in HD affects a nuclear encoded component of Complex II, Complex I, or the process of protein translocation and import into mitochondria.

Over 30 years ago, Mettler showed that sodium azide, a Complex IV (cytochrome oxidase) inhibitor, produced striatal damage and a hyperkinetic movement disorder in primates [87]. More recent animal studies show that striatal injections of mitochondrial toxins produce differential neuronal toxicity identical to that produced by NMDA receptor agonists [88-91]. Several specific inhibitors, such as malonate and 3-nitropropionic acid (3-NP), act at various complexes of the electron transport chain. A naturally occurring plant toxin and mycotoxin, 3-NP, has been associated with neurological illness in animals and humans [92]. 3-NP is an irreversible inhibitor of succinate dehydrogenase that inhibits both the Krebs cycle and Complex II activity of the electron transport chain. Ingestion in cattle causes dyspnea, hindlimb weakness, and motor abnormalities [92,93]. In China, accidental human systemic ingestion of 3-NP from contaminated sugar cane has resulted in neurologic sequelae that include encephalopathy with stupor and subsequent coma. A delayed-onset nonprogressive dystonia with jerk-like movements and facial grimacing results in those patients that recover. Brain imaging has identified bilateral damage to the basal ganglia, particularly the putamen.

In vitro studies have shown that 3-NP reduces cellular levels of ATP and causes neuronal damage by an excitotoxic mechanism [94]. We have demonstrated that systemic administration of 3-NP to both rats and primates can produce selective striatal lesions that are a consequence of secondary excitotoxic mechanisms [95-96]. These lesions accurately replicate a number of motor and neuropathological symptoms observed in HD patients. Systemic administration results in differential sparing of striatal NADPH-diaphorase and large cholinergic neurons with a significant loss of striatal GABAergic neurons. Both enkephalin and substance P striatal neurons, however, are equally affected by 3NP, a finding that is inconsistent with those present in adult-onset HD [97]. 3-NP is the first neurotoxin to show an age-dependent neurotoxicity. The age-dependent neurotoxicity of 3-NP observed in young adult rats in these studies is similar to the typical age of onset in HD. Both freeze-clamp measurements and chemical shift magnetic resonance spectroscopy show that 3-NP impairs energy metabolism in the striatum *in vivo*. While intrastriatal injection of 3-NP results in striatal neuronal death in rats, neurochemical and histologic evaluation shows that markers of both spiny projection neurons (GABA, substance P, calbindin) and aspiny interneurons (somatostatin, neuropeptide Y, NADPH-diaphorase) are equally affected in intrastriatal injections [95].

Interestingly, the lesions produced by intrastriatal injection or systemic administration of 3-NP are blocked by prior decortication, suggesting that intact corticostriatal glutamatergic innervation plays an important role in striatal degeneration produced by systemic administration of 3-NP. As glutamate is a central tenant in provoking excitotoxic cell death in striatal neurons already weakened by the collective molecular events occurring in HD, it is of interest to note that the modulation of other neurotransmitters, such as dopamine, can contribute

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to the neurodegeneration in HD. In addition, the modulation of A2a receptors can ameliorate 3-NP-induced neuronal damage [98]. In primates, chronic 3-NP administration produces selective bilateral striatal lesions characterized by a depletion of calbindin neurons with sparing of NADPH-diaphorase neurons, and proliferative changes in the dendrites of spiny neurons.

Lesion approaches in experimental animals using selective neurotoxins have made it possible to clarify the mechanisms that underlie the hyperkinesis [99-101]. It has been suggested that there is a shift in the pathways of the basal ganglia/thalamocortical circuit in HD [101]. Animals also show both spontaneous and apomorphine-inducible choreiform movement disorders resembling those in HD. These findings clearly associate metabolic stress and striatal neuron vulnerability. It is of interest to note that differences in the periodicity of excitotoxin injections and total dosing levels result in the reported differences in susceptibility. Acute treatments consisting of a single i.p. dose of 3-NP rapidly lead to striatal degeneration within 6–12 h after injection [102-104]. Subacute treatments consisting of daily repeated intraperitoneal injections lead to striatal degeneration over a few days [95,105,106]. Chronic treatments are based on continuous systemic administration of 3-NP (over 5 days to 4 weeks depending on the protocol) using osmotic minipumps implanted subcutaneously. Of great interest is the fact that toxicity in rodents is dependent upon the strain and gender of experimental animal used [107].

Unfortunately, systemic administration of 3-NP is not specific to the CNS alone. There is now very strong data to suggest that 3-NP has severe cardiotoxic effects in addition to neurotoxicity [108]. It may be unclear what the underlying cause of the mortality and clinical phenomena observed in 3-NP toxicity are and to what degree peripheral damage contributes to the clinical and neuropathological outcome measures. As such, postmortem analysis of somatic organs, especially the heart, in assessing pathological involvement is critical in determining the involvement of systemic and neurological contributions to CNS damage. While 3-NP toxicity replicates a number of the cell death mechanisms associated with HD (e.g. electron transport activity inhibition and mitochondrial dysfunction, activation of the apoptotic cascade and execution proteases, increased reactive oxygen species production, and proteolytic calpain activation), transgene and full-length mutant huntingtin rodent models provide the best possible molecular and genetic comparability and pathophysiological correlation to humans with HD. Although toxin models do not help to advance our understanding of the pathogenicity of expanded polyglutamine tracts, this is not to suggest that the 3-NP model is not without merit. The 3-NP model continues to enrich our understanding of specific pathophysiological phenomena in HD associated with excitotoxicity and mitochondrial dysfunction.

Genetic Models of HD

Genetic models have revolutionized the study of human neurological diseases by providing accurate and experimentally accessible systems in which to study molecular pathogenesis. Genetic models also provide an opportunity to test potential treatments and explore their promise for translation to humans experiencing these diseases. The promise of using genetic models for treatment discovery is perhaps greatest in inherited diseases, such as HD, which affect single genes. It must be said, however, that even the genetic models of HD are not perfect, since there are subtle differences in the huntingtin gene from the human orthologue along with dissimilar promoters. In addition, the polyglutamine repeats in some genetic models are quite long and have been suggested to replicate the more fulminant juvenile form of HD and not necessarily the adult-onset form of the disease. The degree of over-expression of mutant protein plays a significant role in the phenotype observed in mice. The gold standard is the human condition and no genetic model replicates all of the findings in HD patients. Each model, however, has valid and useful experimental outcomes that can be used to provide a greater understanding of the disease process in humans and especially in identifying potential therapeutic strategies. While mutant huntingtin has been expressed in a number of invertebrate

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and vertebrate species in modeling different aspects of the HD phenotype, this review will concentrate on mouse models, since they are the most common experimental tool currently in use.

Different genetic mouse lines have been generated with varying phenotypes as a product of how the mutant huntingtin was incorporated into the mouse genome. They fall into three broad categories: (1) mice that express exon-1 or exon-1 and 2 of the human *huntingtin* (htt) gene containing polyglutamine mutations (in addition to both alleles of murine wild-type huntingtin, *Hdh*) [109-111]; (2) mice with pathogenic CAG repeats inserted into the existing CAG expansion in murine *Hdh* (knock-in mice) [112-118]; and (3) mice that express the full-length human *HD* gene (plus murine *Hdh*) [119,120]. Although all of these models share features with human HD, the phenotype of full-length huntingtin mutation models develops gradually over many months and may not have a sufficient expression of disease to use progressive morbidity and survival as endpoints. Although the full-length models are genetically more accurate, the fragment models have a rapidly coursing robust phenotype, well-defined neurobehavioral and neuropathological findings, and die between 3 and 4 months of age. It has been a common practice to use the fragment models for therapeutics research because the outcomes are more clearly established and trials are more easily conducted [121]. The ideal transgenic mouse model should have a robust phenotype, moderate to rapid disease onset and progression, well-defined behavioral abnormalities that can be quantified, and neuropathological features, all of which accurately replicate human HD.

Fragment/Segment Genetic Murine Models of Human HD

Not surprisingly, the degree of similarity to human HD increases the closer the model reproduces the exact neuropathological and molecular conditions for HD. Unfortunately, the more genetically accurate the model is, the more subtle the phenotype. Thus, it has so far been much more feasible to use the fragment models for therapeutics research because the outcomes are more prevalent and definable; hence, trials can be conducted using smaller sample sizes. This category of genetic mice expresses N-terminal fragments of human *HD* and includes the R6/2, R6/1 and N171-82Q lines (Table 1). They show a relatively rapid onset and progression of a phenotype that includes weight loss, motor performance abnormalities, neuropathological sequelae, and a shortened lifespan.

R6/2 Transgenic Mice—The R6/2 line was the first transgenic mouse model of HD and has an N-terminal fragment of htt (exon 1) with approximately 144-150 CAG repeats at exon 1 [109]. It is one of the most widely employed genetic models of HD. The R6/2 model exhibits a progressive homogeneous HD-like phenotype, with survival ranging from 14 to 21 weeks, depending on housing and facility conditions. Differences in survival duration may be the result of variations in housing, handling, environmental enrichment, and the allowable presence of viral and bacterial symbionts, in addition to other factors. Given that enriched environments can alter the progression of behavioral phenotype in R6/2 mice [122], it stands to reason that differences between laboratories may well alter the R6/2 phenotype. This mandates that within any colony of R6/2 mice, a repeatable measurement of survival, or any other phenotypic measurement, is paramount for each successive “f” generation.

Recent findings suggest that the R6/2 HD model exhibits a progressive HD-like behavioral and neuropathological phenotype that more closely corresponds to human HD than previously believed, providing further assurance that the R6/2 mouse is an appropriate model for testing potential therapies for HD [44]. Behavioral analyses of the R6/2 mouse reveal age-related impairments in dystonic movements, motor performance, grip strength, and body weight that progressively worsen until death. There is a very small subset of R6/2 mice, less than 2%, that die prematurely through constant seizure activity (status epilepticus). This may occur as early

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as 60 Days. Over-handling of these mice may exacerbate seizure activity. .Significant neuropathological sequelae, identified as increasing marked reductions in brain weight, are present from 30 days, whereas decreased brain volume and hyperventricular enlargement is present from 60 days, both a hallmark of the disease. In addition, decreased neostriatal volume, striatal neuron atrophy, increased astrogliosis, with a concomitant reduction in striatal neuron number, are present at 90 days of age [44]. Consistent with early adult-onset HD, enkephalin striatal neurons are reduced in comparison to substance P striatal projection neurons [97]. There is, however, equal preservation of enkephalin and substance P striatonigral projections.

Huntingtin-positive aggregates are present at postnatal day 1 and increase in number and size with age, suggesting that disease onset and progression occur before clinical phenomena [44]. The huntingtin inclusions are extensive and found throughout the brain in great numbers, a phenomenon that is inconsistent with that observed in HD patients. It has been suggested that the latter may be the result of using only a portion of the HD gene, transgene effects, and the use of foreign promoters that increase expression levels. There does not appear to be any gender differences in the pathological phenotype. There is remarkable parallelism between the reported mechanisms of disease pathogenesis observed in HD patients and those found in the R6/2 mice, which include altered proteolysis and proteosomal activities, increased protein crosslinking, induced chaperone expression, and defects in vital cellular processes that comprise endocytosis, intraneuronal trafficking, transcriptional regulation, postsynaptic signaling, apoptotic cascades, and alterations in bioenergetic metabolism and mitochondrial function [28,57,123].

Although the R6/2 model has many of the temporal behavioral and neuropathological features observed in HD patients, it is not an exact genetic and neuropathological correlate to HD patients. While some disparity exists in the specific aspects associated with onset and presence of both neuropathological and behavioral alterations observed in the R6/2 mice, with resulting potential confound [124-127], these differences may just be the result from varying methodological practices. Nevertheless, the R6/2 model has a well-characterized progressive phenotype with moderate variability such that experimental groups can contain as few as 10 mice and provide the power to detect 10% differences in many outcome measures. It is possible to perform survival studies, an important potential surrogate indicator for neuroprotection, in approximately 3 months. R6/2 mice have been used in a number of preclinical therapeutic trials [28,57] that have recently been translated to human clinical trials [28,57,123]. The efficiency and clear experimental endpoints of the R6/2 mice remain a major advantage.

It has been long held that synaptic stress may contribute to the neurodegeneration in HD and, as such, modulation of synaptic influences in R6/2 mice, that is glutamatergic and dopaminergic transmission, ameliorates disease progression. Deafferentation of the corticostriatal and nigrostriatal pathways mitigates striatal stress and neurodegeneration. Both surgical and chemical lesions of the corticostriatal and nigrostriatal pathways, respectively, improve the behavioral, neuropathological, and biochemical phenotype in R6/2 transgenic mice and extend survival [128]. Decortication ameliorates hindlimb clasping, striatal neuron atrophy, and huntingtin-positive aggregates, improves *N*-acetyl aspartate/creatine levels, reduces oxidative stress, and significantly lowers striatal glutamate levels. In addition, 6-hydroxydopamine-lesioned R6/2 mice show extended survival along with a significant reduction in striatal glutamate.

There can be great variability in phenotype presentation, which is dependent on CAG repeat size. The number of CAG repeats in the R6/2 line is 148–153 with 500–550 bp, as determined by PCR analysis [44]. An increased number of base pairs greater than 550 results in moderation of the severity of the R6/2 phenotype. With increasing base pair numbers, there is a concomitant increase in CAG repeat size. R6/2 mice in which the base pairs ranged between 600 and 800

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have CAG repeat sizes between 175 and 192. The average survival extension significantly increased in age to 131.4 ± 12.1 days, in contrast to 500–550 bp at 97.4 ± 0.58 days. Base pair numbers of 1,000 and above have CAG repeat sizes consistently above 200, with a mean survival of 147.9 ± 15.6 days. We have found that the variability in survival and the amelioration of the behavioral and neuropathological phenotype in R6/2 mice with increased base pair number and CAG repeat size reduces their utility in therapeutic trials and may confound results [44]. Although great variability in clinical measures is common in human trials, minimizing measurement variability increases the power to detect differences in mouse drug trials. Thus care should be taken to ensure that genetic variability is reduced, providing a relatively homogeneous population of mice within experimental cohorts.

R6/1 Transgenic Mice—The R6/1 mice were developed at the same time as the R6/2 mice and express exon 1 of the human HD gene with approximately 116 CAG repeats. The R6/1 mice have not been as well studied as the R6/2 mice [109]. The R6/1 line has a later age of onset and a slower disease progression. Body weight loss occurs after 22 weeks [129]. They exhibit motor performance abnormalities, as limb clasping, at 4-5 months. There is a significant decline in rotarod performance between 14 and 20 weeks that correlates with the presence of huntingtin aggregates in striatal neurons. There are gait abnormalities and hindlimb clasping similar to the R6/2 mice. These mice can live beyond 12 months. Brain volume is significantly reduced by 18 weeks with striatal neuron atrophy. There is, however, no neuronal loss, as identified by neuron-specific nuclear protein NeuN or met-enkephalin immunostaining in the striatum [129]. Huntingtin aggregates do not appear until 2 months of age. Although it has been reported that both R6/1 and R6/2 mice are resistant to excitotoxic lesions produced by quinolinic acid and malonate [130,131], it is possible that the latter may be attributable to a marked reduction in dopamine levels in the R6/1 mice and R6/2 mice [132,133]. In the R6/1 mice, extracellular dopamine levels are reduced by 70% compared with wild-type littermates, and intrastriatal administration of malonate in these mice resulted in significantly smaller lesion size [134]. In contrast, we have showed that R6/2 mice are more susceptible to the mitochondrial toxin, 3-nitropropionic acid [135]. It may well be that differences in the periodicity of excitotoxin injections and in total dosing levels result in the reported differences in susceptibility. Equally, background strain may play a role in resistance [136]. Of interest to note, reduced sensitivity to excitotoxins may be dependent upon age and CAG repeat length [137].

N171-82Q Transgenic HD Mice—N171-82Q mice have an N-terminal fragment of huntingtin incorporating both exon 1 and exon 2 of the huntingtin gene, with 82 polyglutamines [110]. The N-terminal fragment is driven by the mouse prion promoter and expression is restricted to neurons and no other cells in the CNS. The mice contain two wild-type copies of the gene along with one mutant copy. The phenotype of N171-82Q mice is similar to, but less severe than that found in the R6/2 mice. The first detection of phenotypic abnormality is a failure to gain weight with a significant body weight loss over the last 6 weeks of life. In contrast to the R6 lines of HD mice, seizure activity and hyperkinesis are not present in the N171-82Q mice. These mice show deficits in the radial arm water maze test of working and reference memory at 14 weeks [138]. The commercially available line has a life span range from 130-180 days. The neuropathological features of the N171-82Q mouse models are similar to the R6 mice in that they show striatal atrophy, hyperventricular enlargement, and striatal neuron loss by 16 weeks [139]. While the original description of these mice did not include neuronal loss and astrogliosis, these pathological phenomena have been reported in these mice [139,140]. Huntingtin and ubiquitin-positive inclusions are found as early as 16 weeks and continue to increase in number through end-stage disease, particularly in the pyriform cortex. The phenotype of N171-82Q mice, however, is more variable than that of R6/2 mice and, as such, a greater number of N171-82Q mice are needed for preclinical drug trials within the

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experimental groups (n=20). The N171-82Q mice are a useful model in which to screen drugs using weight loss, performance on the rotarod test, and survival as outcome measures.

Murine Hungtintin Homologue Knock-In Mice

While this group of HD mice may represent a more precise genetic model of HD, the knock-in mice models present with a mild and protracted behavioral and neuropathological phenotype, in contrast to the fragment models. These mice live a normal life span. An expanded CAG repeat is inserted into the murine huntingtin homologue and, therefore, the mutation is genomically correct and under the endogenous Hdh promoter. The mice can be homozygous or heterozygous for the mutation. A number of lines have been made (Table 2). The chimeric HdhQ lines were developed by a mutated exon 1 containing either 111 or 92 CAG repeats. These large polyglutamine repeats cause a CAG repeat instability, predisposing them to subsequent increases in CAG repeat, resulting in a more fulminant disease process [113]. Huntingtin-positive puncta are present in neuronal nuclei by 4.5 months along with an increase in gliosis by 24 months in both of these lines [115]. Mitochondria undergo compensatory changes in calcium sensitivity in both the Q92 and Q111 mice [141]. A knock-in *Hdh* mouse with 72-80 CAG repeats shows aggressive behavior and neuropil aggregates, but no gliosis or neuronal loss [114]. These mice develop rotarod impairment.

The *HdhQIII* knock-in mice have 111 CAG repeats inserted into the murine HD gene [115]. The mice develop a progressive neuropathological phenotype with specificity for striatal neurons, consisting of nuclear localization of the full-length huntingtin protein in medium spiny neurons, and subsequent formation of N-terminal inclusions and insoluble aggregates. The mice eventually develop a late-onset neurodegeneration and gait deficit in older mice at 24 months of age [142]. While there were no differences in rotarod and paw-clasping outcome measures, tunnel walk and stride length revealed a ‘subtle’ gait deficit. Reactive gliosis and toluidine-blue stained striatal neurons were present at 24 months. These neurons were negative for TUNNEL staining, again suggesting a very ‘subtle’ disease progression as reported by the authors.

Mice with greater CAG repeats, however, show a less fulminant phenotype that corresponds to that found in HD patients. A knock-in mouse with 94 CAG repeats has increased rearing at night at 2 months of age, decreased activity at 4 and 6 months, but a normal lifespan. Nuclear microaggregates are present at 4 months and are widely distributed at 6 months of age in these mice. In mice developed by the same group with 140 CAG repeats, a very similar pattern of motor alterations is present [117]. Of interest, disease onset in the 140 CAG mice occurs much earlier than in the 94 CAG mice, consistent with the same phenomena in HD patients. The onset of symptoms in HD patients occurs at a younger age with greater CAG repeat size. In addition, there is increased motor activity and rearing at 1 month of age, hypoactivity at 4 months of age, and a reduction in stride length at 12 months of age. No significant weight loss was observed up to one year of age. Further analysis, however, has shown a much wider range of motor and behavioral dysfunction [143]. Neuronal loss and reactive astrogliosis are not present in the 94 CAG, but found in the 140 CAG mice. The 140 CAG mice show a progressive gliosis, decreases in dopamine and cAMP-regulated phosphoprotein of 32 kDa (DARPP32) (a marker of striatal projection neurons) at 12 months, and neuronal loss at 2 years of age [143]. Huntingtin aggregates are present in both the 94 CAG and 140 CAG mice, occurring much earlier, at one month, in the 140 CAG knock-in mice. With age, there is a more global presence of huntingtin aggregates throughout the CNS. We have noted a derangement in bioenergetic mechanisms, increased oxidative stress, and factors associated with transcription regulation. The 140 CAG mice have reduced ATP brain levels, increased 8-hydroxy 2′-deoxyguanosine, a marker of DNA oxidation, in both brain and urine, and hypoacetylation of histone activities

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[144]. We suggest that the 140 CAG mice may well be useful in premanifest HD investigations as well as in therapeutic evaluation.

Another knock-in mouse model with 150 CAG repeats has late-onset gait abnormalities and develops neuronal intranuclear inclusions predominantly in the striatum [116,118,145]. There is an age-dependent late-onset behavioral phenotype with significant motor abnormalities at 70 and 100 weeks of age measured by rotarod, balance beam, and clasping [118]. At 100 weeks the mice exhibit resting tremor, unsteady movements, and staggering gait. No significant gender differences were observed. There was significant weight loss by 70 and 100 weeks. Gliosis is significantly increased by 14 months. Further analysis of this model showed that striatal nuclear huntingtin and ubiquitin-positive inclusions were initially associated with the matrix compartment by 27 weeks, the topographic striatal area first thought to be involved in HD patients [106]. In older animals, nuclear inclusions were distributed evenly in both the striatal patch and matrix compartments by approximately 2 years, nuclear inclusions were present in most brain areas. Dopamine D1 and D2 receptor binding sites are reduced by at 70 and 100 weeks. Striatal neuron loss was present at 100 weeks, with a 50% loss in striatal perikarya and a 40% reduction in striatal volume. Recent evidence shows NMDA receptor mediated excitotoxicity in this mouse line, providing additional proof that the interaction of huntingtin with NMDA receptors may be an early event in neuronal death in HD [118]. As with the 140 CAG HD mice, the Hdh (CAG) 150 mice may be useful in identifying early mechanisms and premanifest biomarkers of disease.

While the knock-in mouse lines do not have a sufficient expression of disease to use progressive morbidity and survival as an endpoint, they do have a number of measurable neuropathological and behavioral phenotypes that can be validated as potential endpoints in therapeutic studies and may be useful as a secondary model for confirmation. It is clear that a greater investigation into the pathophysiological phenomena of these mice is needed.

Full-length Human HD Gene Transgenic Mouse Models

Transgenic mice with full-length huntingtin containing 48 or 89 CAG repeats have been described that show behavioral abnormalities and neuron loss, but have not been available for use in therapeutic discovery [120]. A yeast artificial chromosome mouse model of HD with the entire human huntingtin gene containing 128 CAG repeats (YAC 128), as well as flanking segments that might involve regulatory elements, develops motor abnormalities consisting of initial hyperactivity followed by difficulty walking along an elevated rotating rod from 6 to 12 months, and then hypokinesis [146,147]. There are other significant motor performance declines starting as early as 3 months that include circling behavior, hindlimb clasping, and gait abnormalities. More recent studies show rotarod and open field abnormalities at 4 and 2 months, respectively [148]. While there is a significant body weight loss in the YAC72 mouse model, there is an increase in body weight at 2 months in the YAC128 mouse by 2 months [148]. Cognitive dysfunction, as measured by a swim test for procedural learning is similar to the perseveration observed in HD patients. The motor deficit in the YAC128 mice correlates with striatal and cortical neuron loss, providing a structural correlate for the behavioral changes [147]. Neuropathological examination shows decreased striatal and cortical volume, reduced striatal neuron area and number by 12 months, the presence of huntingtin immunostaining at 1-2 months, huntingtin macraggregates starting at 12 months and increasing in number by 18 months in the YAC128 model [148]. The YAC 128 model has been used extensively to investigate pathogenic mechanisms of HD and most recently to identify therapeutic strategies. Medium-sized spiny striatal neurons are more vulnerable to NMDA receptor-mediated death in the YAC transgenic mouse model of HD expressing full-length mutant huntingtin [149]. Both mitochondrial and apoptotic pathways are altered in these mice [150]. The neuropathology in the YAC mice has excellent fidelity with human HD. As with the 140 CAG

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and 150CAG mice, the length of time for phenotype development may hinder the use of this model for treatment paradigms unless early biomarkers of disease can be identified in the YAC mice.

New transgenic mouse, created using a bacterial artificial chromosome (BAC), look promising [151]. These mice have a 240 kb BAC that contains the entire 170 kb human huntingtin locus. These mice show progressive motor deficits. They exhibit a significant reduction in rotarod performance at 2 months with worsening behavior through 12 months. The BACHD mice aso show a significant gain in body weight, similar to the YAC 128 mice, that plateaus at 12 months. By 12 months there is marked gross brain atrophy and brain weight loss, with significant decreases in both cortical and striatal volume. Degenerating dark neurons were present in the striatum at 12 months without significant neuronal loss. Huntingtin inclusions were detected at 12 and 18 months, almost entirely within the cortex and a few small inclusions within the striatum. Electrophysiological studies show that sponrtaneous excitatory transmission is significantly reduce in the BACHD mice, suggesting early abnormalities in cortical input to striatal neurons. Although these transgenic mice expressing a full-length mutant huntingtin have not yet been used in a published therapeutic discovery experiment, there are efforts underway.

A transgenic rat model also exists that expresses 51 human CAG repeats [152]. Expression of the gene is under an endogenous rat promoter. The rat is normal at birth and by 24 months has significant body weight loss. There is a progressive poor performance on rotarod after 2 months of age, along with cognitive deficits by 12 months [153]. Gait abnormalities and head dyskinesias occur starting at 10 months. The neuropathological sequelae include hyperventricular enlargement along with huntingtin inclusions present throughout the brain.

Lentiviral-Mediated Mutant Huntingtin Model

A lentiviral delivery of mutant huntingtin has been developed in a rat model using 44, 66, and 82 repeat fragments [154]. There is an increased expression of ubiquitinated htt aggregates starting one week after injection with great numbers of inclusions by 4 weeks time. Striatal neuron degeneration, loss of DARPP-32 staining, and cell death occur over 6 months. The neurodegeneration is specific to GABAergic neurons, with little or no effect on striatal interneurons, consistent with observations in HD patients.

Non-Human Primate Models of HD

Both toxin and genetic primate models of HD exist and play an important role in extending those findings observed in rodent models HD [66,69,99,100,101,155]. While the toxin lesions provide an experimental model that closely parallels the neuropathological, neurochemical, and clinical features of HD, a recently developed transgenic HD rhesus monkey model that expresses expanded huntingtin polyglutamine shows clinical phenomena that include dystonia and chorea characteristic of that found in HD patients [155]. While marked expression of huntingtin aggregates were present in the brains of those monkeys that succumbed to an early death, no striatal neuron degeneration was present. As HD is a slowly progressive disorder, it may be years before these monkeys show a full HD neuropathological phenotype. Clinical follow-up continues with surviving monkeys. As such, the availability of non-human primates with HD could be valuable in any final analysis evaluating the most promising therapeutic candidates for HD patients.

Biomarkers of Huntington's Disease

A major goal of current clinical research in Huntington's disease is to improve early detection of disease and premanifest detection of neuronal dysfunction with translation to therapeutic

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trials. Biomarkers are urgently needed for diagnosis, disease progression, and for potential disease-modifying therapies that are being developed and evaluated in clinical trials, especially at the preclinical stage. The development of early premanifest biomarkers is of great importance, as these may improve the power and cost-effectiveness of drug trials. As the mechanism of pathogenesis in HD is not yet clear, biomarkers will be required at all stages of disease and will necessitate multiple combinations of detection methods. One complementary research strategy has been to perform parallel correlative biomarker studies in HD patients and animal models of HD and make comparisons. This approach takes advantage of advances being made in animal models to best understand the most effective therapeutic strategies. While many different approaches have been undertaken to identify biomarkers, profiling objective biomarker measurements of HD has proven somewhat difficult at the present time. There has been, however, some advancement in this arena, particularly in R6/2 mice. It has been shown that biomarkers of protein crosslinking are significantly elevated in both HD patients and in HD mice. Both transglutaminase and gamma glutamyl lysine activities are significantly increased in brain samples in a disease-dependent manner [156]. There is also evidence that biomarkers of nucleosomal and bioenergetic dysfunction are present in both HD mice and HD patients, representing biomarkers as predictors of disease onset and progression [57]. Lastly, 8-hydroxy-2-deoxyguanosine, a marker of DNA oxidation, is significantly increased in brain and urine from HD patients and HD mice [144,157]. Of great interest are studies using high-dose creatine in HD patients and in HD mice, showing parallelism in biomarkers of disease pathogenesis and treatment efficacy. Creatine treatment significantly improves brain creatine levels and reduces urine levels of 8-hydroxy-2-deoxyguanosine, a marker of DNA oxidation, in both HD patients and mice [144,157]. While it has not yet been confirmed that therapeutic experiments demonstrating improved phenotype in HD mice are predictive of benefits in humans, the above evidence demonstrates that mice do provide value. Correlative biomarker analysis in both mice and man will provide a more rapid and desirable body of evidence to fully inform clinical decision-making, and identify what factors contribute to the equipoise in determining whether preclinical mouse information about a therapy makes clinical study warranted in patients. While the efficiency and clear experimental endpoints of the R6/2 mice remain a major advantage, the fulminant and early expression of disease in these mice may not allow for premanifest and early symptomatic identification of biomarkers. Full-length knock-in HD mice, however, may provide the best possible molecular and genetic comparability to human HD and strengthen the ability to confirm the detection of biomarkers, especially prior to disease onset, given their slow disease progression.

Methodological Considerations for Mouse Therapeutic Trials

Although making the therapeutic leap from genetic models to humans fulfills one of the promises of molecular medicine, it also brings risk to human subjects and expends human and financial resources. Thus, how data from experiments using experimental models are used to inform choices about clinical trials requires reflection and examination. Among the important issues are what constitutes an informative genetic model, what principals should be followed in designing experiments using these models, what body of evidence is desirable to fully inform clinical decision making, and what factors contribute to the equipoise in determining whether preclinical information about a therapy makes human study warranted.

While toxin models play a role in understanding mechanisms of excitotoxocity and mitochondrial dysfunction in HD, they fall short as a reliable model of an autosomal dominant genetic disease characterized by the misfolding of the mutant huntingtin protein. Toxin models are acute experimental paradigms in contrast to slowly progressive events that occur over years in HD patients and months and years in genetic models of HD. As such, this section will focus on issues related to standardization of experimental therapeutics in transgenic mouse models of HD and discuss some of the salient issues related to translation from mice to humans.

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Genetic animal models of inherited neurological diseases provide an opportunity to test potential treatments and explore their promise for translation to humans experiencing these diseases. Therapeutic trials conducted in mouse models of HD have identified a growing number of potential therapies that are candidates for clinical trials and have been reviewed in multiple publication venues [28,57]. While it is very exciting to have these candidates, there has also been increasing concern about the feasibility and desirability of taking all of the compounds that may work in mice and testing them in patients with HD. There is a need to begin to prioritize leads emerging from transgenic mouse studies, however, it is difficult to compare results between compounds and laboratories and there are also many additional factors that can affect translation to humans.

Genetic models encompass a variety of assays in which the mutant gene or some portion of it has been introduced into living organisms which experience a phenotypic response in some measure indicative of the human disease. Screening of high-throughput assays using libraries containing thousands to millions of small molecules can bring candidate therapies forward based upon obtaining a desired effect on a biochemical or cellular phenotype, irrespective of knowledge about mechanism of action. However leads arise, proof of efficacy in mammalian models is considered a requisite before considering possible testing in humans. For compounds already in the pharmacopoeia it can be easy to contemplate rapid translation to clinical trials. For compounds that are not yet pharmaceuticals or nutriceuticals, efficacy in mammalian models would lead first to standard preclinical pharmacokinetic, toxicity, teratogenicity studies, and perhaps the development of more promising analogs before testing can begin in humans. When selecting compounds for study in preclinical experiments, we have prioritized compounds already in human use because of the indispensable knowledge base that comes with them and the existence of pharmaceutical material ready for human use.

Transgenic mouse models of HD have been extensively used in the last several years to test potential therapies [28]. While most studies have concentrated on compounds that have already had human use, we are also beginning to test leads arriving from screens performed in cellular and submammalian models. Some of the compounds that we have demonstrated to be efficacious in transgenic mice have gone on to clinical testing, often in trials we have also participated in designing [57]. Through this experience and discussions with many others, we have developed a perspective about using transgenic mouse experiments to stimulate and guide clinical trials. We have also encountered a wide range of thoughts in the HD research community, from the opinion that preclinical studies in mouse models are of limited significance because mice and humans are so different, to suggestions that there should be rigid criteria for success in the mouse models before a therapy is considered for human clinical trials. It is quite complex to consider what constitutes sufficient data from mouse models to justify translation to humans. The lack of a proven neuroprotective therapy for HD also effects the equation by keeping the level of urgency high.

Though there is a need to begin to prioritize leads emerging from transgenic mouse studies, it may be difficult to compare results between compounds and laboratories. There are also many additional factors that can affect the overall desirability of compounds, such as availability from a manufacturer, expected toxicity, redundancy of mechanism, and the nature of potential interactions with other agents. As yet, there have been insufficient fully powered clinical trials in humans to permit a comparison of therapeutic benefits in the two species from any given therapy. Thus it has not yet been confirmed that experiments demonstrating improved phenotype in transgenic mice are predictive of benefits in humans. Similarly, it is unknown whether the magnitude of benefit in mice predicts the magnitude of benefit in humans. Nevertheless, results from human clinical trials will illuminate the value of mouse clinical trials in the foreseeable future. In the meantime, there are clinical trials being planned, a growing

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list of therapies that are effective in mouse models, and a need for discussion about how transgenic mouse therapeutic trial data can best be used.

Outcome Measures

As in human clinical trials, the use of appropriate methodology in mouse trials determines whether the experimental outcomes are valid and useful. Many of the same considerations apply both to human and mouse trials but are not always considered in the latter. For example, blinding of study personnel about the treatment condition of the animals they are studying should be standard practice. A *priori* power analysis is necessary to establish whether the study has sufficient numbers of mice for each endpoint. Whereas in human trials enormous variability is a given; in mouse trials it is possible to minimize the variability and thereby increase the power to detect smaller differences in smaller groups. It is important to understand the genetic, physical, and environmental sources of variability to take advantage of this.

An estimation of the sample size is a critical first step. The numbers of mice for preclinical studies must be carefully considered and reflect the different outcome measures to be analyzed. Using power analysis, our experience in survival studies shows that an 'n' of 10 in the R6/2 transgenic mice and an ‘n’ of 20 in the N171-82Q transgenic mice in each experimental group are necessary to have an 80% chance of detecting a 30% difference between groups. Our experience with neurohistopathological measures and neurochemical assays has shown that a standard deviation of 15-20% can be expected. A similar power analysis suggests that to detect a 30% difference between groups an "n" of 10 is necessary for each cohort. Others have provided similar power analyses in detecting differences in huntingtin accumulation over time in R6/2 mice [159]. A 30% reduction in huntingtin aggregation is observed at 95% confidence using 10 R6/2 transgenic mice per group. Power calculations also show that the probability of detecting a deficit in R6/2 mice on the running wheel and climbing cage was greater than 90% using a group size of 14 mice (160). In the full-length human mutant huntingtin bacterial artificial chromosome (BAC) transgenic mouse model, power analyses were performed to estimate the number of mice to rescue disease-relevant outcomes (151). Power analysis showing a 30% rescue of rotarod deficit at 6 months would require 22 BAC mice. In addition power analysis showed that a sample size of 14 BAC mice was necessary to detect a 30% rescue of dark neurons, along with 5 and 11 BAC mice, respectively for a 30% rescue of cortical and striatal volumes. A 15-20% standard deviation could be expected.

As in human clinical research, it is important to have inclusion and exclusion criteria, which should be predetermined. For example, there is good reason to consider excluding “runts” (we routinely exclude mice weighing less than 8 gms at 21 days), mice with injuries, mice in which CAG repeat length is out of the median range (CAG repeat must be monitored in the colony), and mice unable to perform some of the planned evaluations, such as the rotarod. Treatment groups should be physically and genetically comparable. In particular, there should be a systematic assignment of mice to experimental groups to prevent overrepresentation of sibs in any group. For example, it can be predetermined that no more than one or two mice from any litter be in a given experimental group. Mice should be evenly distributed into cohorts by body weight. The environment in which these mice are housed and treated needs to be uniform, since environmental enrichment slows disease progression in HD mice and may be considered a therapeutic treatment in and of itself [161]. Another important consideration is the onset and duration of treatment in mouse trials. Initiation of treatment at weaning, as has been most common, is analogous to treating presymptomatic humans. Initiation of treatment once a motor or other clinical phenotype is evident would be analogous to most human trials for HD, the latter of which have generally used early symptomatic subjects.

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Primary Measures—There are many potential outcome measures that can be used in mouse therapeutic trials. These bear close examination because they can differ enormously in relevance with some clearly being much more informative and specific than others. For clarity, in neurodegenerative disease research we are especially concerned with achieving neuroprotection, which at its most basic level is the preservation of neuronal processes, somata, and function. Measures that assess these directly (brain weight, gross atrophy, cellular atrophy, neuronal counts, gliosis, volumetric imaging) should be considered the primary outcome measures. While it may also be possible to model the treatment of symptoms in genetic models and it may also happen that improving symptoms corresponds to neuroprotection, assessments of symptoms should be considered secondary outcome measures. The reason for this is that symptoms can be modified without affecting neurodegeneration, for example by modifying motivation, level of consciousness, energy, etc. This distinction is important to keep in mind when considering how informative the results of a mouse therapeutic trial might be for considering translation to humans. A treatment cannot be considered to be neuroprotective in mice in the absence of neuropathological evidence that brain atrophy, cellular atrophy, or neuronal loss has been prevented. The quality of neuropathology in mouse therapeutic trials is thus of foremost importance. It should also be mentioned that other neuropathological measures, such as protein aggregate load or the expression of molecules of interest, are only meaningful if brain atrophy, cellular atrophy, or neuronal loss are measured to provide a context for interpreting them.

In our labs, cohorts of treated and untreated wild-type and mutant littermate mice, are euthanized at early, moderate, and late stages of their phenotypes for neuropathological analysis. Standardization of tissue processing is critical to the analysis. As an example, cut tissue sections mounted from water will expand and those mounted from phosphate buffer will contract, resulting in entirely different brain measures. Brain specimens are identified by the originally assigned code so that the subsequent studies are performed blind to the investigator as to the genetic identity and treatment of the mice. Quantitative methods are essential as observation alone can only detect large differences and semi-quantitative methods are prone to many types of errors. We use stereology to obtain various brain and cellular volumes and counts and also to quantify other pathological features, such as huntingtin aggregates.

Secondary measures—Secondary outcome measures include survival, body weight, performance on motor and cognitive tasks, and laboratory studies examining toxicity or putative mechanisms of action. These should be completed at the same time of day because of diurnal and circadian rhythms. Mouse survival, or a surrogate for mouse survival, which are usually criteria for advanced morbidity that agrees with an institution's animal care policies and triggers euthanasia, is an especially useful secondary outcome measure. Besides generally correlating well with neuropathology, it provides a relevant measure of the magnitude of benefit that is both understandable and enables ready comparison with other therapies. A dose versus survival study is recommended, using at least three doses demonstrating no significant efficacy, significant efficacy, and significantly greater morbidity and/or mortality in mutant mice. It is important to observe mice in any treatment paradigm twice daily throughout the experiment for any signs of morbidity and mortality. Strict criteria are necessary to determine when mice should be euthanized. While this may vary between institutions, our own criteria for euthanization is the point in time when the mice are unable to initiate movement and right themselves after being placed upon their side and gently prodded for 30 seconds. Two independent observers confirm these criteria.

While body weight has often showed early and significant improvements in some mouse therapeutic trials, little effect has also been observed in other trials despite dramatic improvement in survival. Our practice is to measure body weight weekly, on the same day, usually from the time of weaning. Diurnal feeding habits necessitate that weights be taken at

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the same time, morning or afternoon. As mice progress towards the latter stages of disease, we usually increase the frequency of weighing to twice weekly.

A variety of motor tasks have been used in mice to assess the clinical phenotype. These include measuring activity and grooming, the length of time a mouse can balance on a rotating rod, grip strength measurement, and gait. Preserved motor performance correlates with improved survival and neuroprotective outcomes, though compounds administered to the CNS can also have pharmacological affects that independently modify motor performance. The rotarod test, in which mice are required to walk along an elevated rotating rod, is generally accepted as the most sensitive and encompassing and is widely used. This equipment is commercially available and its use has been standardized within most laboratories and can be compared across experimental studies. In the fragment models, we perform testing twice weekly. There are a number of variations of rotational speed and whether the rod is at a constant speed or accelerating which investigators have applied. The length of time that the mice remain on the rotating rod is used as the measure of competency on this task. Mice are tested until they are unable to perform. On any given day, mice may fail or refuse to perform this task. If this occurs early on in the time course of disease phenotype, additional repeated trials the next day may be useful to ensure that performance failure is a reflection of disease. In the absence of a rotarod apparatus, the onset of forelimb and hindlimb clasping, a measure of neurological abnormality in HD mice, can be assessed daily until identified and confirmed by a second investigator.

Declining cognition occurs early in HD and the mouse models are expected to develop cognitive deficits as well. However, few cognitive assessments have been validated in the mouse models currently in use. In the fragment models, it is difficult to complete training before the phenotype has already compromised performance. Nevertheless water maze, habituation to acoustic startle, and prepulse inhibition have been used by a number of investigators and efforts to develop further cognitive tests, such as serial reaction time and other implicit memory tasks are ongoing [162,163]. Cognitive endpoints may prove especially valuable in the full-length models in which motor symptoms are slow to develop.

Open Field Testing, which assesses general activity, is a complex measure that may be most useful in the full-length models that progress more slowly. Commercially available units to detect movement can analyze and quantify multiple motor behaviors. In our laboratory, testing is usually performed during the dark phase of the diurnal cycle when mice are most active. Measurements can be made during the first 15-30 minute interval after placement in a novel open field. Horizontal and vertical motions can be counted and locomotor activity, rearing, circling, and grooming assessed. The open field box must be cleaned between mice to prevent behavioral effects from the odors of previous occupants. Testing is performed every other week in the R6/2 and N171-82Q models and once a month in the full-length models to avoid habituation to the open field and to ensure novelty of the measured behavior. There can be some specific differences in the patterns of behaviors observed. For example, some HD mice seem to continually repeat an abortive grooming process.

A behavioral observation system, SHIRPA, is a three stage, semi-quantitative protocol designed to characterize phenotype changes in mice. In therapeutic trials, behavioral and functional changes are scored by an observer blinded to treatment [164]. A composite score is calculated following the assessment. A modified SHIRPA has been developed for longitudinal use in mice with neurological disease [165]. The utility of such a behavioral protocol is in its standardization and may be a useful cross measure for all laboratories performing therapeutic drug trials in mice with neurological disease. The scoring, however, requires detailed scrutiny of some behaviors and function in HD mice that are subject to interpretation, such as limb clasping and physical appearance. For this reason, it is also important to maintain observer consistency throughout a study.

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Pharmacokinetic Studies—Several types of laboratory studies contribute crucial information that can greatly enrich the preclinical information about a compound. Pharmacokinetic analysis of the study compound, its metabolites, or of molecules expected to be affected by the treatment, help to insure that the compound reaches the brain and performs as expected. Pharmacokinetic studies are useful in combination drug trials to examine the effects on bioavailability of one compound in relation to the other compound. Additionally, understanding effective doses in mice, along with blood and brain levels, can set the stage for asking whether such doses and levels can be achieved in humans. In our experience, 0.5-1.0 ml of blood is required to produce sufficient serum for testing levels of agents and it is collected by cardiac puncture, requiring euthanization. HPLC with electrochemical detection along with spectrometric analysis are the methods of choice [156,166]. Because pharmacokinetic studies provide critical information regarding optimal dosing, our current approach has been to perform maximum-tolerated-dose studies to determine the LD50 dose starting at a minimum tolerated based upon previously available ADMET data and increase the dose one fold b.i.d. each administration over the course of 14 days or until LD50 is reached. In addition, we employ a pharmacokinetic model to identify a series of concentration-time data points in blood, urine, and brain. The bioequivalence is evaluated by comparison of variables such as peak concentration, time of peak concentration, elimination half-life, and area under the concentration curve in each tissue sample. These experimental studies are completed in both wild type and mutant mice prior to a drug trial in mice and allow us to determine the optimal pharmacokinetic parameters for dosing the mice. In addition to pharmacokinetic studies, it can be very helpful to perform pharmacodynamic studies aimed at determining whether a compound's expected mechanism of action is actually working. Both brain and peripheral body tissue samples are used in the analyses. This can be important as many compounds have multiple potential mechanisms of action. We currently assess nucleosomal (histone acteylation and methylation) and bioenergetic (ATP/ADP/AMP, creatine, phosphocreatine, and coenzyme Q_{10} and Q_9 levels) dysfunction, along with 8-hydroxy-2-deoxyguanosine concentrations, a marker of DNA oxidation. Each of these biomarkers of disease is significantly altered in both central and peripheral tissue samples [57]. It is additionally important to determine whether any of the treatments might work by suppressing expression of the huntingtin transgene, since this might help the mice but not humans in the absence of identical regulation. This is especially important in mouse lines in which expression is regulated by exogenous promoters and for drugs that affect aggregate formation and gene transcription [167]. Transgene expression can be analyzed using Western and Northern assays, as well as real time-PCR methods.

Therapeutic Strategies—Many potential therapies have now been tested in genetic models of HD and some have been demonstrated to be neuroprotective in HD transgenic mice (table 1). Efficacy in mouse trials has provided the rationale for a number of clinical trials that have already occurred or are planned. These include coenzyme Q_{10}, creatine, remacemide, riluzole, minocycline, ethyl-epa, phenylbutyrate, and cysteamine [57]. Most of these have been early phase clinical trials, so the predictive value of the mouse therapeutic trials are not yet known. However, because these are actually very good models at many levels of analysis, it would be surprising if some compounds benefiting the mouse models did not prove to benefit humans with HD. Since clinical trials may be expensive, effortful, and years in length depending on the type, there is growing discussion within neurodegenerative disorder clinical trials organizations, such as the Huntington Study Group and sponsors, about how much preclinical information is enough and how to prioritize among many efficacious compounds. Of course the bar should be higher for later phase trials and higher when the interventions have greater potential for risks to human subjects.

The impact therapeutic trials in genetic models can have on selecting compounds for clinical trials in humans depends on many factors relating to the quality and breadth of the preclinical data, as well as on the potential risks and benefits to performing the clinical trials. In this review,

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we have discussed the many factors that affect the quality of an individual mouse therapeutic trial. Of course, the highest quality studies and those with the most robust neuroprotection should have the greatest impact. Neuropathological evidence of neuroprotection remains the most important finding with all other outcomes potentially providing additional support. Since the numbers of compounds demonstrating some efficacy continues to grow, an issue of growing importance is the question of how to compare their efficacy to better prioritize them. Since procedures differ between labs, since the models have many differences, and since even the same mouse line can have different phenotypes in different labs because of genetic drift, background strain differences, and/or the presence of microbes within individual vivaria, it is difficult to compare the magnitude of one treatment's effect with another's. We would propose that laboratories performing therapeutic trials in mouse models use positive control compounds to help place their results in context. For example, several labs have replicated benefits with cystamine, so including a cystamine treatment cohort in trials of more novel therapeutics could help calibrate results [57].

As in human clinical trials, the use of appropriate methodology in mouse trials determines whether the experimental outcomes are valid and useful. Many of the same considerations apply to both human and mouse trials but are not always considered in the latter. Based upon our experience, positive outcomes in mice are critical indicators in determining whether compound efficacy provides sufficient information to fully inform human clinical-trial decision-making. While there are many different outcomes that could be used in characterizing drug efficacy, the following minimum guideline criteria are essential to strengthen the evidence for drug translation to patients.

1. Blinding of study personnel about the treatment condition of the animals they are studying should be standard practice.
2. Power analyses are necessary to establish whether the study has sufficient numbers of mice for each endpoint.
3. Use of both fragment and full-length HD mouse models in preclinical drug trials once the potential of a compound has been established. Our current standard measure has been the R6/2 with confirmation in the 140 CAG HD mice.
4. As in human clinical research, it is important to have inclusion and exclusion criteria in order to exclude bias (eg. CAG repeat size, body weight, parental inheritance) Treatment groups should be genetically comparable to prevent overrepresentation of sibs into any experimental cohort.
5. A dose *versus* survival study is recommended, using at least three doses, to decide whether therapeutic benefits stabilize (saturate) or decline past the optimum dose determined by pharmacokinetic studies.
6. Confirmation of experimental findings in at least one independent laboratory.
7. Rotarod Test: 20% and 15% improvement in R6/2 and 140 CAG mice, respectively.
8. Open Field Test: 20% and 15% improvement in R6/2 and 140 CAG mice, respectively.
9. Survival extension of at least 15% in R6/2 mice. Survival is defined as meeting the criteria for euthanasia in accordance with the institution IACUC guidelines.
10. Neuropathological measures that include a 20% reduction in gross brain and ventricular measurements, a 25% reduction in huntingtin aggregate load, and a 30% reduction in neuronal cell atrophy and/or neuronal loss using unbiased stereology.

11. At least one pharmacodynamic or biochemical measure that correlates globally with phenotypic progression and neuroprotection in mice and humans. We use 8-hydroxy-2-deoxyguanosine concentrations, as they are elevated in urine and brain in both HD patients and HD mice. This measure can be developed to the specific compound in use.

There is a growing body of evidence suggesting that the phenotypes from mouse models of neurological diseases closely correlate with human diseases and may validate known CNS drug targets in a therapeutically relevant manner. The strengths of the HD mouse models are in their utility to provide parallel pathophysiological targets that are present in HD patients, in their potential as sensitive predictors for therapeutic intervention, and their promise in the development of novel drug agents. While drug trials in mice confirm therapeutic direction, the challenge is in determining what dose might be of value in patients since the pharmacokinetics of mice and man is dissimilar.

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Table 1

N-terminal Fragment Models of Human HD

Rodent Model	CAG repeat	Behavioral changes	Neuropathology	Survival	References
R6/1 mouse	116	Significant body weight loss at 22 weeks. Motor performance abnormalities at 4-5 months.	Reduced brain volume by 18 weeks. Neuronal atrophy but not neuronal loss is present. Huntingtin aggregates are present at 2 months. Reduced. dopamine levels.	12+ months	97,119
R6/2 mouse	144-150	Dystonia with limb clasping by 6 weeks. Body weight loss by 8-9 weeks. Reduced hindlimb and forelimb performance by 9 and 11 weeks, respectively. Rotarod changes by 5 weeks. Seizures. Diabetes	Significant brain weight loss at 30 days. Gross brain atrophy by 60 days with hyperventricular enlargement. Progressive neuronal atrophy with neuronal loss by 90 days. Progressive huntingtin aggregate formation starting at day 1. Astrogliosis at 90 days. Reduced dopamine levels.	12-18 weeks	97,111
N171-82Q mouse	82	Body weight loss Motor performance deficits, and limb clasping by 11 weeks. Water maze deficits at 14 weeks.	Gross brain atrophy, hyperventricular enlargement, striatal neuron atrophy and loss, and huntingtin aggregate presence by 16 weeks.	130-180 days	98,126,127, 128
Transgenic Rat	51	Progressive rotarod decline by 2 months, with significant body weight loss by 24 months. Cognitive deficits by 12 months. Head dyskinesias by 10 months.	Hyperventricular enlargement and huntingtin inclusions. Striatal neuron loss.	none reported	134,135

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Table 2

Knock-in Mouse Models of HD

Rodent Model	CAG repeat	Behavioral changes	Neuropathology	Survival	References
Hdh/Q72-80	72-80	Aggressive behavior. No weight loss. Rotarod impairment.	No neuronal loss or reactive astrogliosis. Huntingtin aggregates by 28 weeks with nuclear aggregates by 96 weeks	normal life span	102
HdhQ111	109-111	Gait impairment by 96 weeks.	Diffuse huntingtin activity by 6 weeks with nuclear inclusions by 48 weeks and neuropil aggregates by 68 weeks. Astrogliosis present by 96 weeks.	normal life span	103
CAG140	140	Body weight loss. Initial hyperactivity at 4 weeks with increased hyperactivity by 12 weeks. Gait abnormalities by 48 weeks.	Nuclear and neuropil, aggregates are present by 8 months, with diffuse huntingtin activity starting by 2 months. Neuronal loss.	normal life span	105,129
CAG150	150	Body weight loss Motor performance deficits on rotarod, gait, and beam balance by 70-100 weeks.	Striatal huntingtin immunoreactivity at 28 weeks. Nuclear inclusions at 37 weeks. Reactive astrogliosis at 56 weeks. Loss of striatal neuron perikarya and volume by 100 weeks in homozygous mice.	normal life span	104,106