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NBD special issue on **Vectorology**

**Viral-mediated overexpression of mutant huntingtin to model HD in
various species**

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Abstract

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expansion of CAG repeats in the huntingtin (Htt) gene. Despite intensive efforts devoted to investigating the mechanisms of its pathogenesis, effective treatments for this devastating disease remain unavailable. The lack of suitable models recapitulating the entire spectrum of the degenerative process has severely hindered the identification and validation of therapeutic strategies. The discovery that the degeneration in HD is caused by a mutation in a single gene has offered new opportunities to develop experimental models of HD, ranging from *in vitro* models to transgenic primates. However, recent advances in viral-vector technology provide promising alternatives based on the direct transfer of genes to selected sub-regions of the brain. Rodent studies have shown that overexpression of mutant human Htt in the striatum using adeno-associated virus or lentivirus vectors induces progressive neurodegeneration, which resembles that seen in HD. This article highlights progress made in modeling HD using viral vector gene transfer. We describe data obtained with of this highly flexible approach for the targeted overexpression of a disease-causing gene. The ability to deliver mutant Htt to specific tissues has opened pathological processes to experimental analysis and allowed targeted therapeutic development in rodent and primate pre-clinical models.

Introduction

Huntington's disease (HD) is a progressive and devastating neurodegenerative disorder characterized by choreiform movements, cognitive deficits and psychiatric dysfunction (Vonsattel et al., 1985). This autosomal dominant hereditary brain disorder is caused by the presence of multiple CAG repeats in exon 1 of the huntingtin (Htt) gene, which encode an abnormally long stretch of glutamine residues. This mutation leads to neuronal degeneration and cell loss, most prominently in the striatum (The Huntington's Disease Collaborative Research Group, 1993). Post-mortem analysis shows ubiquitinated intranuclear inclusions, suggesting abnormal processing/folding of the polyglutamine (polyQ) domain in affected cells. However, the pathogenic mechanisms leading to neurodegeneration have yet to be discovered. HD affects approximately 1 in 10,000 people worldwide (Myers et al., 1993) with its symptoms generally beginning at age 40-50 and having a mean duration of 15-20 years. Since the identification of the gene mutation responsible for HD, important advances have been made in understanding the disease, but much is still unknown and fundamental questions remain.

HD as a monogenic disorder can serve as model for the study of other more common neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease. These disorders all share features including: delayed onset; selective neuronal vulnerability despite widespread expression of disease-related proteins during the entire lifetime of the individual; abnormal protein processing and aggregation; and cellular toxicity involving both autonomous and cell-cell interaction mechanisms. To develop effective treatments and understand HD pathogenesis, the development of animal models is critical. Since the cloning of the Htt gene and identification of the mutation as a CAG expansion in the region that encodes the N-terminal part of the protein, numerous *in vivo* genetic models have been developed. Although transgenic models reproduce the typical signs of early HD, current models do not fully recapitulate all the features of late stages of the pathology, including the severe striatal degeneration and the some motor and cognitive deficits observed in HD patients. Consequently, despite the information gained from such models, critical questions concerning HD pathogenesis and disease progression, and their relationship to the motor and cognitive symptoms typical of HD remain largely unanswered.

The emergence of highly efficient viral vectors for CNS applications offers an approach to overcoming some of these limitations. Currently, gene transfer into the brain is a versatile and potent tool for neuroscientists, which is widely employed to decipher the mechanisms implicated in neurodegenerative diseases. It has been proposed that these very same vectors be used to overexpress disease-causing genes and develop new models of neurodegenerative diseases. Different vectors have been extensively studied but lentiviral and adeno-associated viral vectors have emerged as the most promising candidates. This review will discuss the progress made so far in gene transfer-based models of HD.

Huntington's disease, a model for CNS disorders

HD can be considered as a prototypical model of a monogenic, fully penetrant neurodegenerative disorder characterized by an accumulation of misfolded protein. The Htt gene was

discovered 18 years ago and has greatly accelerated our understanding of the pathogenesis. The disease is inherited in an autosomal dominant manner with age-dependent penetrance (Langbehn et al., 2004). Individuals at risk of inheriting the expanded CAG repeat can be identified before clinical onset of the disease by predictive genetic testing. Formal diagnosis of HD is made on the basis of characteristic extrapyramidal motor signs of chorea (Huntington Study Group, 1996). Generally, clinical symptoms develop very rapidly after onset and consist of motor symptoms characterized by hyperkinesia, which evolve to hypokinesia with bradykinesia, rigidity and dystonia. Typically, the earliest motor signs are eye movement abnormalities, followed by the progressive appearance of orofacial dyskinesia, involving the head, neck, trunk and arms before becoming chorea (Brouillet et al., 1999). Cognitive dysfunction includes dementia characterized by difficulties in retrieving memories, slowed information processing and cognitive inflexibility (Cummings, 1995; Lawrence et al., 1996). Psychiatric disturbances most commonly manifest as apathy and depression (Wang and Qin, 2006). The most striking neuropathological feature of HD is marked atrophy of the caudate and putamen within the basal ganglia. In addition, the neuronal loss in the striatum is accompanied by pronounced gliosis (Vonsattel et al., 1985). Interestingly, many studies have consistently shown that the degenerative process does not equally affect all striatal cells, but preferentially affects GABAergic, medium-sized spiny neurons; (Cicchetti and Parent, 1996; Ferrante et al., 1987; Ferrante et al., 1985; Kowall et al., 1993). Although the striatum is the most profoundly affected region, neuronal loss may also occur in the cortex, thalamus, substantia nigra, hypothalamus and deep cerebellar nuclei. Since the genetic cause of HD was uncovered in 1993, several hypotheses have been put forward to explain the toxicity of mutant Htt, including excitotoxicity, oxidative stress, impaired energy metabolism, and abnormal protein-protein interactions, which in turn may cause transcriptional dysregulation (Crook and Housman, 2011). The cellular functions of wild-type Htt are still only partially understood (Cattaneo et al., 2005). Htt shuttles into the nucleus, has a role in vesicle transport, and can regulate gene transcription or RNA trafficking. Furthermore, Htt is necessary for early embryonic development. Most available evidence, including its dominant genetic transmission, the presence of abnormal protein aggregates, and the findings of biochemical, cell, and mouse model studies, suggests that HD arises predominantly from the gain of a toxic function produced by the abnormal conformation of mutant Htt (Shao et al., 2008; Tobin and Signer, 2000). Much of what we know about HD biology arises from the study of model systems.

Modeling Huntington's disease

Cellular models. Cell lines are valuable for biochemical investigations and are particularly suitable for transient, stable or inducible expression strategies. On the other hand, primary neurons or mixed cultures reproduce some cell-cell interactions, though not all the complexities of neuronal circuits, and these cultures have been extensively used to study disease pathogenesis and therapeutic screening. One can notably mention a number of *in vitro* models based on cells of neuronal origin (Lunkes and Mandel, 1998), transfected neuronal cell lines (Cooper et al., 1998; Liu, 1998), primary neurons derived from transgenic HD mouse models (Petersen et al., 2001; Saudou et al., 1998), and striatal cells isolated from knock-in mice (Trettel et al., 2000). However, in some of these *in vitro* models the

expression of mutant Htt does not lead to cell death (Petersen et al., 2001), whereas others generate cell yields insufficient for extensive experimentation (Saudou et al., 1998). Recently, induced pluripotent stem cells were derived from HD patients to investigate mutant Htt-related phenotypes such as toxic effects or changes in cell metabolism (Park et al., 2008).

Invertebrate models: Drosophila and Caenorhabditis elegans expressing mutant Htt proteins display progressive behavioral changes and neuronal degeneration. These features facilitate the study of molecular pathogenesis and provide a rapid initial screen of potential therapeutic interventions (Bates and Hockly, 2003). Jackson et al. created an HD model in *Drosophila* by expressing truncated wild-type and mutant forms of Htt and demonstrated that extensive polyQ expansion led to repeat length-dependent nuclear aggregation and severe and age-dependent degeneration (Jackson et al., 1998). However, the extent to which these models recapitulate HD biology is still uncertain.

Mammalian models: The first animal models of HD were generated through the use of toxins (McGeer and McGeer, 1976). These early studies focused mainly on mitochondrial impairment and excitotoxicity-induced cell death, which have been described in HD brains (Beal et al., 1993; Brouillet et al., 1995). In 1996, a transgenic mouse model was generated by introducing a fragment of a juvenile HD patient's Htt gene into the mouse genome (Mangiarini et al., 1996). This model revealed that the expression of exon 1 alone, containing a CAG expansion, was sufficient to produce some of the neuropathological features of HD. To further address specific pathways in the cascade of pathogenic events, new transgenic mice and rats have been created (Gray et al., 2008; Hodgson et al., 1999; Schilling et al., 1999). These HD models were produced by overexpressing either gene fragments of (N171-82Q mice, short stop, Exon 2) or the entire (BACHD and YAC models) human Htt gene carrying the expanded CAG repeats, and also by knocking the human Htt gene into the corresponding mouse gene locus (Levine et al., 1999; Lin et al., 2001; Menalled et al., 2002; Shelbourne et al., 1999; Wheeler et al., 1999). These models share behavioral deficits such as motor symptoms assessed by the rotarod test, electrophysiological alterations, striatal neuron atrophy, the presence of intranuclear inclusions, and altered transcriptional profiles. However, clear differences exist among the various transgenic models and some cardinal features of HD pathology are poorly reproduced, including severe neuronal degeneration and reduced life span (reviewed in Crook and Housman, 2011). Mouse models expressing N-terminal fragments of Htt have the most robust and rapidly progressive phenotypes and thus have been frequently used for therapeutic trials. Mice overexpressing full-length Htt generally present more subtle phenotypes but exhibit neurodegeneration that may be somewhat more selective than other models. These models have been used to study the mechanisms that lead to mutant Htt protein cleavage or the early stages of HD pathogenesis (Gray et al., 2008; Slow et al., 2003).

Viral-mediated gene transfer

Various viral vectors have been developed and utilized for transferring genes into different organs, including the brain (Déglon and Hantraye, 2005; Kay et al., 2001; Kirik and Bjorklund, 2003; Lundberg et al., 2008). In fact, the CNS has been used as a target to demonstrate the potential of these vectors to transduce cells that have no or very low mitotic activity. The recombinant viral vectors

utilized for CNS gene transfer studies have been derived from adeno-associated virus (AAV), lentivirus (LV), adenovirus (Ad), and herpes-simplex virus (HSV). HSV vectors were the first gene transfer approaches investigated for the brain (Federoff et al., 1992; Geller and Breakefield, 1988) and were followed by adenoviral vectors (Horellou et al., 1994; Le Gal La Salle et al., 1993). However, the efficiency and persistence of transgene expression, as well as the vector safety profiles, all improved significantly with the subsequent advent of AAV (Kaplitt et al., 1994; McCown et al., 1996) and lentiviral vectors (Blomer et al., 1997; Naldini et al., 1996).

Recombinant AAV vectors have a cloning capacity of roughly 6 kb, and the only viral elements remaining in the recombinant vector are the inverted terminal repeats (ITR) in the distal ends of the viral genome, which are structures required for helper virus-mediated replication and capsid packaging. Following infection, AAV vectors support transgene expression in post-mitotic cells for the lifetime of the individual (Mandel et al., 2006). The only serotype so far utilized in CNS clinical trials has been AAV type 2, which has displayed an excellent safety profile (Lim et al., 2010; Mandel et al., 2006). Recently, other serotypes have become available (Chao et al., 2000). Many of these new AAV vectors, such as AAV1 (Passini et al., 2003; Wang et al., 2003), AAV5 (Davidson et al., 2000), AAV8 (Dodiya et al., 2010), AAV9 (Van der Perren et al., 2011) and AAV10 (Sondhi et al., 2007) display greater transduction efficiency, greater diffusion in the brain parenchyma, and higher levels of transgene expression than AAV2 (Mandel et al., 2006).

LV vectors pseudotyped with the VSV-G protein have become some of the most widely used vectors for biological research and functional genomics, and this gene transfer technology has now reached the clinic (D'Costa et al., 2009; Manilla et al., 2005; Schambach and Baum, 2008). LV belongs to a subclass of retroviruses that integrate into the host cell genome, which results in persistent expression of the gene of interest. However, in contrast to retroviral vectors, LV vectors efficiently transduce non-dividing cells. Moreover, the capacity of LV vectors to accommodate relatively large transgenes (up to 9 kb) is an advantage over AAVs. First generation LV vectors, based largely on human immunodeficiency virus 1 (HIV-1), included multiple components of the HIV genome. Most of these elements have since been removed in the newer generation versions (Zufferey et al., 1998). Recently, vectors with mutations in the integrase gene were developed to prevent vector integration into the host genome and further improve the safety of the system (Banasik and McCray, 2010). Furthermore, improvements in LV vector technology have facilitated large-scale production of recombinant LVs by using stable packaging cell lines (Farson et al., 2001; Klages et al., 2000). VSV-G-pseudotyped LVs enable transgenes to be locally expressed in neurons because they do not undergo anterograde or retrograde transport. This feature is of interest for studying the relationship between the regional localization of disease gene expression and resulting behavioral deficits. In contrast, AAV vectors lead to more widespread expression. In some cases, anterograde and retrograde transport of viral particles can be exploited to replicate pathologies that affect large areas of the brain (Hollis et al., 2008; White et al., 2011). Recently, several groups have investigated whether viral vectors can be used to develop new animal models of neurodegenerative disorders.

Viral vectors to model CNS disorders

This strategy holds various advantages compared with classical transgenic approaches. Viral vector gene transfer can produce local and massive overexpression of a disease gene. This provides a rapid, highly flexible and cost-effective, *in vivo* paradigm to study the impact of pathogenic gene expression during CNS development or in adult animals. When the viral vector is injected in adults, it avoids the emergence of compensatory mechanisms that may protect the brain from disease-induced cell death in transgenic animals. The high transduction efficiencies, as well as ability to deliver robust and sustained transgene expression, can lead to the rapid appearance of functional and behavioral abnormalities and severe neurodegeneration. Transduced animals can be their own internal control if transgene expression is limited to one hemisphere. Multiple genetic models can be created in a short period of time. Finally, models can also be established in different mammalian species including non-human primates (NHP), thereby providing opportunities to assess complex behavioral changes and perform longitudinal follow-up of neuropathological alterations by imaging.

Targeted injections of viral vectors into different brain areas can be used to investigate the regional specificity of a neuropathology and eliminate potential side effects associated with widespread overexpression of the transgene. In addition, the respective contributions of neuronal and non-neuronal cells, which have each been shown to play important roles in neurodegenerative diseases, could be addressed with vectors that target specific cell types. By using different AAV serotypes, which differ in the receptors utilized for cell entry, or by pseudotyping LVs with rabies or mokola viral envelope proteins, the transduction efficiency of certain cell types may be significantly altered (Wong et al., 2004). VSV-G-pseudotyped LV and AAV2 have strong neurotropisms (Davidson et al., 2000; Naldini et al., 1996). However, LV having an astroglial tropism have been obtained by changing the envelope used for the pseudotyping, inserting a tissue-specific promoter and taking advantage of microRNA post-transcriptional regulation to suppress residual transgene expression in undesired cells (Colin et al., 2009). This vector offer new opportunities to dissect the contribution of glial cells in CNS pathologies such as HD (Faideau et al., 2010). For AAV vectors, astrocyte targeting was successfully achieved with AAV1 and AAV5 vectors containing transgenes controlled by glial promoters (Davidson et al., 2000; Wang et al., 2003). However, glial cell-specific promoters, such as GFAP, do not necessarily preclude transgene expression from being detected in neurons (Jakobsson et al., 2004). Conversely, ubiquitous promoters, such as the CMV promoter/enhancer, do not necessarily produce high transgene expression levels in all cell types (Cannon et al., 2011; Jakobsson et al., 2003). The characterization of new cell-type specific promoters and vectors with various tropisms will be essential to further expand tool kit available to model CNS pathologies. For diseases, such as Canavan's disease, in which a role for oligodendrocytes has been implicated, efforts are still needed to develop vectors that predominantly target these cells.

Modeling HD by overexpressing expanded polyglutamine tracts *in vitro*

LV vectors have been used to express mutant human Htt protein with extended glutamine repeats in primary cultures of striatal and cortical neurons. One advantage of this approach is that it provides a large amount of material to perform biochemical analyses to explore the mechanisms

underlying striatal death in HD. Indeed, >90% of the cells are infected with these vectors and express the transgene for up to eight weeks (Zala et al., 2005). The *in vitro* proof-of-principle was performed with lentiviral vectors expressing a mutant Htt protein (htt171-82Q) under the control of mouse phosphoglycerate kinase 1 (PGK) promoter to generate a chronic model of HD in rat primary striatal cultures (Zala et al., 2005). Mutant Htt produced a slowly progressing pathology characterized by the appearance of neuritic and nuclear aggregates at one month, followed by neuronal dysfunctions and cell death between six and eight weeks. Cortical cultures infected with the same LV-Htt171-82Q vector showed no sign of neuronal dysfunction despite accumulation of numerous inclusions, which agrees with the selective vulnerability of GABAergic neurons observed in HD patients. This progressive striatal pathology contrasts with the rapid appearance of cytoplasmic and nuclear aggregates in HEK293 cells 16 hours after being infected with an AAV2 vector expressing a GFP fusion protein gene containing 97 CAG repeats (CMV promoter) (Senut et al., 2000). The slow progression of the pathology in the lentiviral model, compared with other *in vitro* models, provides an extended time window to dissect the cascade of events leading to striatal cell death and to test experimental therapies.

Owing to the high cloning capacity of adenovirus (E1-deleted, synapsin promoter), several Ad vectors expressing mutant or wild-type versions of full-length Htt or N-terminal truncated Htt were generated and tested (Huang et al., 2008). In primary striatal cells isolated from E17 mouse embryos, the formation of Htt inclusions was considerably delayed in cells expressing mutant full-length Htt compared to cells expressing Htt171-128Q. The subcellular locations of these proteins also differed, as the mutant full-length Htt protein formed neuritic and cytoplasmic inclusions and the truncated Htt fragment was found in nuclear aggregates.

To determine whether the Htt disease-gene overexpression reproduced the transcriptional changes seen in HD, microarray analyses were performed on the LV-Htt171-82Q model (Runne et al., 2008; Zala et al., 2005). Expression of a wild-type Htt fragment (Htt171-18Q) or a control transgene (enhanced GFP) caused only a small number of RNA changes in primary neuronal cultures. The transcriptomic effects of mutant Htt were time and polyQ expansion-length dependent, and occurred in parallel with other manifestations of polyQ toxicity over 4–8 weeks. Specific changes caused by LV-mediated Htt171-82Q expression in striatal cells were differential detection of 20% of all mRNA probe sets, which accurately recapitulated those observed in human patients. HD-related transcriptomic changes were also observed in primary neurons expressing a longer fragment of mutant Htt (Htt853-82Q) and included gene sets involved in neuronal signaling, neuron differentiation, protein degradation and RNA processing. Notably, the transcriptomic concordance coefficient was equivalent to the highest-scoring transgenic mouse models of HD (Kuhn et al., 2007) and demonstrated that these striatal transcriptome dysregulations could be attributed to the intrinsic effects of mutant Htt (Runne et al., 2008).

Benchoua and collaborators took advantage of this *in vitro* model to dissect the involvement of mitochondrial complex II defects and the role of dopamine in the selective vulnerability of striatal neurons (Benchoua et al., 2008; Benchoua et al., 2006). Finally, the LV *in vitro* HD model has been used to assess the potential therapeutic benefit of trophic factors and chaperones (Perrin et al., 2007;

Zala et al., 2005).

Lentiviral- and AAV-mediated delivery of mutant huntingtin to model HD *in vivo*

The first *in vivo* study in adult rats was performed using an AAV vector for expressing a GFP fusion protein containing a long polyQ tract (Senut et al., 2000; Table 1). Intrastratial injection of this vector caused intracytoplasmic and ubiquitinated intranuclear aggregates to form rapidly in neurons. This study demonstrated that expression of an Htt polyQ tract throughout life does not necessarily induce cell death, but rather that acute overexpression of a polyQ tract in adult neurons is sufficient to induce a pathology. In a subsequent study, VSV-G pseudotyped LV vectors encoding the first 171, 853 or 1520 amino acids of wild-type Htt containing only 19 glutamine repeats or encoding mutant forms with polyQ tracts of 44, 66 or 82 residues were produced (de Almeida et al., 2002). The Htt mutants induced a pathology characterized by the rapid appearance of either neuritic or nuclear ubiquitinated Htt aggregates, or both, followed by neuronal dysfunction and astrogliosis. These abnormalities finally led to robust and selective degeneration of medium-sized striatal GABAergic neurons after 12 weeks while the large cholinergic interneurons and GABAergic aspiny interneurons were spared. In agreement with previous studies of transgenic mice and of presymptomatic patients, this model showed that aggregation occurs before ubiquitination (Gomez-Tortosa et al., 2001; Lin et al., 2001; Mangiarini et al., 1996). Moreover, a temporal and spatial analysis of the striatum revealed that the length of the Htt protein modulates the pathogenesis and that the onset of the pathology inversely correlates with the number of CAG repeats. The number of Htt aggregates was significantly lower for the longer Htt fragments.

Régulier and collaborators used tetracycline-regulated lentiviral vectors to generate high expression levels of a large Htt fragment corresponding to the first 853 amino acids and induce an early pathological onset (Regulier et al., 2003). The expression levels reached with these vectors were 4- to 5-fold higher than those produced by the vectors containing the constitutive PGK promoter and exacerbated the pathology. Overexpression of the TRE-853-82Q protein was associated with a loss of the GABAergic marker DARPP-32, the proteolytic release of N-terminal Htt fragments and translocation of mutant Htt to the nucleus as early as one month post-injection. Similar results were obtained in a transgenic mouse model with inducible expression of full-length mutant Htt (Tanaka et al., 2006), which yielded nuclear accumulation of a 60-kDa N-terminal cleavage product. This fragment is smaller than calpain- or caspase-derived Htt cleavage products, but it is comparable to a product, termed cp-A, which accumulated in the nuclei of cells in a previously described cell model (Lunkes et al., 2002).

The tetracycline operator is not only a strong promoter leading to high transgene expression levels but also a system that allows transgene expression to be suppressed by peripheral administration of doxycycline (Furth et al., 1994; Kistner et al., 1996). Such conditional expression of mutant Htt represents a powerful means of exploring the relationship between mutant protein expression and progression of the disease. Blockage of mutant Htt expression revealed that continuous expression of the mutant protein is required for maintaining inclusions and for progression of the clasping phenotype (Yamamoto et al., 2000). In the LV vector-based model, turning off Htt853-

82Q expression for two months was sufficient to restore DARPP-32 expression and to clear Htt aggregates from the striatum of adult rats. Altogether, these data demonstrate the reversibility of Htt-mediated neuronal damage, which is a key feature favoring the development of effective therapies and for determining the time-window of intervention.

Gene transfer also may be valuable to target a subpopulation of cells and explore its function in the pathology. The notion of a non-neuronal component of neurodegenerative disease is becoming increasingly regarded as an important aspect of the disease process (Lobsiger and Cleveland, 2007). Colin et al. developed a lentiviral vector selectively targeting astrocytes with the G protein envelope of the Mokola lyssaviruses which displays a preferential tropism toward astrocytes (Colin et al., 2009) combined with a detargeting strategy based on a neuronal miRNA to eliminate residual expression in neuronal cells. In a subsequent work, this tool was used to evaluate the contribution of astrocytes in HD (Faideau et al., 2010). They injected a lentiviral vector expressing the first 171 amino acids of the human Htt with normal or expanded glutamine repeat into the striatum of adult mice. At 4 weeks post-injection, mHtt aggregates were observed in astrocytes and not in neurons, confirming the selectivity of the vector. Astrocytes expressing mHtt progressively developed a reactive phenotype and showed a marked decreased expression of glutamate transporters and a reduction of glutamate uptake. The impairment in glutamate transport and recycling capacity was associated with a significant decrease in the expression of two neuronal markers, DARPP32 and NR2B in this model. Moreover, they showed that selective gene transfer of GLT-1 into striatal astrocytes partially rescues the phenotype. Thus, the ability to selectively infect non-neuronal cells may be a key component when developing gene therapy based therapeutics for neurodegenerative disease.

Recently, Franich and collaborators characterized a new model of HD generated using an AAV1/2 vector expressing exon 1 of mutant Htt containing 70 CAG repeats (controlled by an NSE promoter) (Franich et al., 2008). Quantitative RT-PCR revealed that the level of Htt exon 1 expression in the striatum of adult rats was >100-fold higher than the level of endogenous Htt, which explained the rapid-onset and severe striatal pathology. By comparison, the expression level produced by the LV-Htt171-82Q vector was 25-fold higher than the level of endogenous Htt (Drouet et al., 2009). The transduction efficiency and diffusion of the vector throughout the rat striatum appeared to be substantially greater with AAV1/2 vectors than with LV vectors and was similar to that reported recently using an AAV1/8 vector in mice (DiFiglia et al., 2007). However, striatal neurons were not uniformly transduced with this AAV1/2 vector. It transduced high numbers of cholinergic interneurons, leading to toxicity and neuronal cell death (2-5 weeks), irrespective of the transgene expressed (wild-type, mutant Htt or EGFP). Furthermore, robust expression of mutant Htt was detected in the globus pallidus and the substantia nigra, produced as a result of axonal transport of AAV particles (Chamberlin et al., 1998; Senut et al., 2000). Anterograde transport of AAV has been suggested in NHPs, following injection of the caudate or putamen with AAV1, 5 or 8 vectors encoding GFP (Dodiya et al., 2010). Cy3-labelled AAV1 and AAV9 capsids are anterogradely transported along axons in mice, and transgene expression was detected in synaptically connected areas (Cearley and Wolfe, 2006; Cearley and Wolfe, 2007). Retrograde transport was observed with AAV1 and AAV5 in the brain (Burger et al., 2004). For LV vectors, VSV-G pseudotyped LV vector particles were not transported,

whereas retrograde transport were observed for LV particles containing a rabies envelope protein (Kato et al., 2007; Kato et al., 2011; Mazarakis et al., 2001). These axonal transport properties could be exploited to express mutant Htt in distal structures of the basal ganglia, including the cortex, where large pyramidal neurons that project to layers V, VI and to a lesser extent, layer III, are preferentially lost in HD.

The large volumes of distribution of newly identified AAV serotypes favor the study of behavioral deficits. Motor dysfunctions measured by increases in clasping frequency, footslips during beam walking and spontaneous exploratory forepaw movements (cylinder test) have been observed in animals injected with AAV1/2- and AAV1/8-based Htt expression vectors (DiFiglia et al., 2007; Franich et al., 2008). Moreover, the percentage of clasping days positively correlated with the extent of neuronal death observed in the striatum (DiFiglia et al., 2007). In a recent study, Hult and collaborators showed that local overexpression of Htt853-79Q or Htt171-79Q (AAV2/5 vector) in the hypothalamus was sufficient to recapitulate metabolic disturbances, namely the endocrine abnormalities that accompany the obesity observed in BACHD transgenic mice (Hult et al., 2011). To demonstrate that obesity was due to hypothalamic dysfunction, AAV5 vectors expressing the Cre recombinase were used to selectively inactivate the mutant Htt gene in the hypothalamus. This study illustrated the potential of combining gene transfer with transgenic animals to study how mutant Htt disrupts various pathways and the complex circuitry involved in the pathogenic process.

Viral vectors to create HD models in large animals

A large animal HD model would be particularly valuable for the development and validation of new therapeutic strategies. NHPs are highly similar to humans in terms of neuroanatomy (organization of the basal ganglia), motor behavior (movement repertoire) and cognitive behavioral characteristics (Brouillet et al., 1999). In fact, a variety of abnormal movements highly reminiscent of those seen in HD patients have been observed in NHPs (Hantraye et al., 1990). Thus, whereas rodent studies are highly informative for initial exploratory studies, primate studies are necessary for producing results with better therapeutic relevance and higher predictive validity. Another advantage of primate models is that non-invasive and high resolution imaging of brain structures through MRI or PET can be used to determine the anatomopathological changes associated with HD symptoms and thus can improve our understanding of how the degenerative process progresses (Hantraye et al., 1992).

Initially, specific toxins were employed to generate HD models in primates. While the massive cell death produced in the ibotenic-induced excitotoxic lesions (Hantraye et al., 1992) or 3-nitropropionic acid (3-NP) models (Brouillet et al., 1995) make them good for studying neuroprotective therapies for HD, it makes them inefficient for assessing the progressive nature of the disease (Roitberg et al., 2002). In addition, there are no clear associations between the mechanisms implicated in the generation of HD-like symptoms and the genetic cause of HD. Notably, these models lack the Htt cytoplasmic inclusions that are a pathological hallmark of the disease. Although the chronic 3-NP model reproduces many phenotypic features of HD, including a frontal-type syndrome resembling the cognitive deficit associated with HD, it does not replicate the early events of HD pathogenesis that are specifically triggered by mutant Htt (Mittoux et al., 2000; Palfi et al., 1996).

Thus there is a need for a genetic model of HD in primates in order to develop and validate new pre-clinical approaches for HD. Recently, our laboratory scaled-up the LV vector-based modeling in NHP in order to recapitulate the behavioral and neuropathological features of HD and to evaluate therapeutic strategies in a large animal model (Palfi et al., 2007). LV Htt171-19Q or Htt171-82Q vectors were delivered in four tracks located in the pre-commissural, commissural and postcommissural sensorimotor region of the dorsolateral putamen. This particular region was targeted to replicate the motor abnormalities observed in presymptomatic HD patients and in primates following excitotoxic striatal lesions. Similar to what has been observed in rats, lentiviral-mediated delivery of Htt171-82Q but not Htt171-19Q led to the formation of neuritic and nuclear ubiquitinated aggregates, followed by neuronal dysfunction and astrogliosis (Table 1). Unilateral injections of LV Htt171-82Q induce chorea following apomorphine stimulation at nine weeks post-injection. When delivered bilaterally, spontaneous chorea occurred at 16 weeks and continued for up to 30 weeks. These data demonstrate that the local production of mutant Htt is sufficient to produce spontaneous choreic movements in primates. Overexpression of mutant Htt in the dorsolateral putamen reproduced a spectrum of basal ganglia motor deficits typical of HD, including hand, leg and head dyskinesia, leg dystonia, and even tics. Furthermore, behavioral analysis of these animals showed that their neurological deficits progressed in manner similar to the progression seen in HD patients. Initially, an early pre-symptomatic stage appears that can be revealed by the administration of the dopamine agonist apomorphine, and is followed by a symptomatic phase characterized by spontaneous choreiform movements (Klawans et al., 1980). Following our study, Chan and collaborators published the first transgenic monkey model of HD (Yang et al., 2008). This remarkable achievement resulted from the combined use of lentiviral gene transfer and improvements in transgenic technology. LV vectors expressing exon 1 of the human Htt gene with 84 CAG repeats under the control of the ubiquitin-C promoter were injected into the periventricular space of rhesus monkey oocytes. Among the five newborns, one survived for a month and exhibited dystonia and chorea two days after birth. Unfortunately, most of the monkeys died during the early post-natal period and couldn't be evaluated for behavior (Chan and Yang, 2009; Yang et al., 2008).

Altogether, these studies with viral-mediated expression of mutant Htt provide a robust *in vivo* genetic model for selective neural degeneration that will facilitate future studies on the pathogenesis of cell death and enable pre-clinical screening for HD therapeutics.

From viral vector-based models to potential therapeutic strategies for HD

These studies support the use of viral vector-mediated gene transfer as a platform for testing new therapeutic strategies for this fatal disorder. Currently, HD patients are given general symptomatic and non-specific treatments such as antichoreic drugs with tetrabenazine (dopamine antagonist) or neuroleptics (Ross and Tabrizi, 2011). However, several clinical trials with drugs for the treatment of HD have been initiated recently (Munoz-Sanjuan and Bates, 2011; Walker, 2007). In addition, disease-modifying treatments are being investigated that may lead to disease-altering therapies. Also being considered are therapeutic strategies aimed at counteracting cellular perturbations and/or those that directly target polyQ protein production, cleavage or conformation (Ross and Tabrizi, 2011;

Zuccato et al., 2010).

Using either *in vitro* cellular models or *in vivo* rodent models of mutant Htt toxicity, we demonstrated that brain aging (Diguët et al., 2009), the JNK pathway (Perrin et al., 2009), and the phosphorylation state of mutant Htt and histone H3 are playing a role in polyQ toxicity (Colin et al., 2005; Martin et al., 2011; Pardo et al., 2006). In addition, we have evaluated trophic factors CNTF and BDNF (Escartin et al., 2006; Escartin et al., 2007; Zala et al., 2005), the transcriptional regulator CA150 (Arango et al., 2006) and chaperones (Perrin et al., 2007) for neuroprotective effects. However, the most attractive and promising strategy for the treatment of HD is certainly RNAi. Gene-silencing techniques that target polyglutamine-encoding mRNA and block or reduce the production of mutant Htt protein, have the potential to halt or, at least, delay the process of neuronal death and therefore constitute a promising strategy for HD. Several studies using viral-mediated delivery of short hairpin RNAs (shRNAs) have led to reduced Htt transcript levels, decreased formation of inclusions and improvements in behavioral tests in HD animal models (Franich et al., 2008; Harper et al., 2005; McBride et al., 2008; Rodriguez-Lebron et al., 2005). We have recently demonstrated that coincident silencing of the normal endogenous wild-type Htt could be considered as therapeutic strategy (Drouet et al., 2009).

In parallel, we are currently investigating a mutant Htt allele-specific silencing technique, which would represent the ultimate solution to blocking polyQ pathogenesis while preserving wild-type Htt expression and functions. Single nucleotide polymorphisms (SNPs) have been used to discriminate wild-type and mutant transcripts of polyQ-based disorders (Alves et al., 2008; Miller et al., 2003; Xia et al., 2004). For HD, SNPs in exons of the Htt gene have been described, and genotypes as well as allele frequencies have been reported (Lombardi et al., 2009; Pfister et al., 2009; van Bilsen et al., 2008). However, appropriate HD animal models are needed to validate this approach *in vivo*. Indeed, knock-in mice do not express human Htt (Menalled, 2005) and transgenic models expressing short N-terminal fragments of human Htt, such as R6/2 (Mangiarini et al., 1996) and N171-82Q (Schilling et al., 1999), and viral-based HD models (de Almeida et al. 2002; DiFiglia et al. 2007; Franich et al. 2008) do not contain the corresponding SNPs. Finally, transgenic mice expressing full-length Htt possess just one human allele (Gray et al., 2008; Slow et al., 2003), which allows efficacy to be analyzed, but not selectivity, which is an important aspect of the present strategy. To overcome this issue, we have developed new HD models based on LV vector-mediated expression of chimeric mutant Htt reporter system that consists of the sequence of the first 171 amino acids of the mutant human Htt fused to the Htt exons containing the SNPs (Figure 1; Ruiz et al. unpublished data). These chimeric constructs produce a striatal neuropathology similar to the original vector encoding Htt171-82Q (Ruiz et al., poster 859.13 at the Society for Neuroscience meeting, San Diego, 2010).

Perspectives

The efficiency of gene transfer vectors for transduction of the CNS has improved remarkably recently, leading to safer vectors and long-term, robust transgene expression in the brain (Lundberg et al., 2008; Mandel et al., 2006; Manfredsson and Mandel, 2011; Taymans et al., 2007). These improvements, coupled with a progressive understanding of the pathological mechanisms of neurodegenerative diseases, have led to the initiation of phase I/II clinical trials with AAV and LV

vectors for the treatment of PD and AD (Eberling et al., 2008; Kaplitt et al., 2007; Marks et al., 2010; Tuszynski et al., 2005).

In parallel, proof-of-principle studies have demonstrated the usefulness of these vectors not only for the delivery of therapeutic candidates but also for the development of HD models in rodents and primates (Déglon and Aebischer, 2002; Déglon and Hantraye, 2005; Kirik and Bjorklund, 2003; Lundberg et al., 2008). One drawback to these approaches is the necessity to perform these experiments in biosafety level 2 laboratories, which are not present in every research center, particularly those focused on behavioral studies. Nevertheless, several groups have now successfully applied vector-mediated expression to model familial forms of neurodegenerative diseases such as Alzheimer's disease (AD; APP expression), Parkinson's disease (PD; α -synuclein expression) and spinocerebellar ataxia type 3 (SCA3; ataxin-3 expression) (Alves et al., 2008; Kirik et al., 2003; Lawlor et al., 2007). In these studies, the local overexpression of mutant genes was associated with severe neuropathologies that recapitulated some of the specific features of these disorders (de Almeida et al., 2002; Kirik et al., 2003; Lawlor et al., 2007; Palfi et al., 2007).

Infection of a limited number of cells, or a subtype of cells, is particularly suited for dissecting the contribution of specific circuitry and pathways in these pathogenic processes. Recently, efforts have been devoted to design and characterize cell-specific promoters (Brene et al., 1994 and 2000; Mayford et al., 1996; Glover et al., 2002). One can notably mention the pleiade promoter project, a consortium initiative aiming at producing a large panels of promoters for selective expression in the brain (Portales-Casamar et al., 2010). As alternative to tissue-specific promoters, one can combine gene transfer approach, the Cre/loxP system and transgenic mice for deleting endogenous or exogenous genes in selective cells or brain areas. Several studies demonstrated that Cre recombinase could be effectively delivered to discrete regions of the adult mammalian brain using AAV and LV vectors (Ahmed et al., 2004; Pfeifer et al., 2001; Scammell et al., 2003). Using this approach, Hult and collaborators showed that hypothalamic inactivation of the mutant Htt gene (flanked by loxP sites) with a Cre recombinase-expressing AAV5 vector prevented the development of the metabolic phenotype in BACHD mice (Hult et al., 2011). Another strategy to capitalize on the large number of Cre transgenic mouse lines is to produce Cre-inducible viral vectors with a double-floxed inverted open reading frame (ORF). In these viral vectors, the gene of interest is cloned in the antisense orientation and is flanked by two pairs of incompatible lox sites (loxP and lox2722). Upon transduction in Cre transgenic lines, Cre-expressing cells invert the transgene ORF in a stable, irreversible fashion and thereby activate transgene expression under the control of a strong, constitutive promoter present in the viral vector (Gradinaru et al. 2009).

If these different strategies are interesting to dissect the molecular mechanisms implicated in neurodegenerative disorders, a widespread expression of a mutant gene is required to closely recapitulate features of neurodegenerative diseases. Several studies have explored methods to transduce most brain regions, by optimizing delivery methods or volume and rate of infusion, by identifying viral vectors that diffuse efficiently throughout the CNS and by injecting the vectors during the neonatal period to exploit the smaller size and immaturity of the brain. The intraventricular injection of new AAV serotypes having anterograde and retrograde transport properties into neonatal mice

resulted in widespread, high-level expression of the transgene (Broekman et al., 2006; Passini et al., 2003). Recently, Kornum et al., demonstrated that a single injection of AAV5 in the striatum of one-day-old rat pups transduces more than one million striatal cells, as well as transducing input and output areas, large parts of the cortex, substantia nigra, septum, hippocampus, and thalamus (Kornum et al., 2010). Combining new AAV serotypes and neonatal injection, not only in rodents but also in species closer to humans, should better recapitulate the spectrum of symptoms seen in patients.

Developing such well-suited animal models of HD will greatly facilitate studies of higher-order cognitive function, a devastating feature of the pathology and will be critical for evaluating new therapeutic strategies for this fatal disorder.

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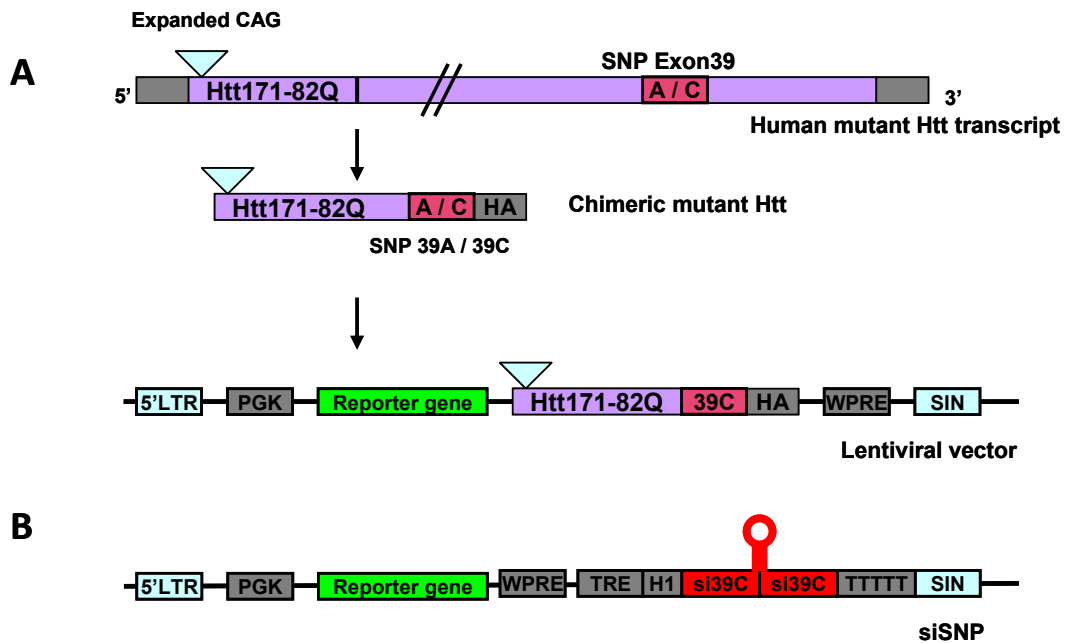


Figure 1. Schematic representation of the viral vector encoding chimeric mutant Htt. We choose SNP within the human mutant htt transcript: exon 39 (rs363125). The example of chimeric htt with exon 39 is illustrated: the sequence surrounding the SNP (A or C) is fused in frame to the 5' sequence of mutant htt encoding the first 171 amino acids. A sequence encoding the HA tag is added at 3' the end to ensure the detection of all fusion proteins. The fusion construct is then cloned in a SIN transfert vector.

(B) Representation of lentiviral vector expressing the siSNP (example of si39C). The shRNA is cloned downstream of a Tet responsive element (TRE) and a H1 promoter in the 3'LTR of the vector. A second expression cassette contains a GFP reporter gene under the control of a PGK promoter. The SNP was located at the position 10 from the 5' end of the guide strand of the siRNA.

Table 1. Summary of published reports using lentiviral and AAV vectors to model HD by overexpression of mutant Htt.

First author Year	Vectors (promoter)	Construct: Htt fragment CAG repeats	Species	Injection site	Neuropathology	Behavior	Observations
Senut 2000	AAV2	Htt exon1 13/97Q	Rats	Striatum	Cytoplasmic/nuclear aggregates+ubiquitin 12d Cellular apoptosis 12d	-	Nuclear aggregates in SN+VTA
DiFiglia 2007	AAV1/8 (CBA)	Htt400 18/100Q	Mice	Striatum	Loss striatal neurons Intranuclear Htt aggregates in striatal+cortical neurons Neuropil aggregates striatum 2w	↑claspings 14d Deficits on beam walking 7d	Colesterol conjugated siRNA targeting Htt ameliorated neuropathology and behavior
Franich 2008	AAV1/2 (NSE)	Htt exon1 20/70Q	Rat	Striatum	Intranuclear inclusion 2w Neuronal death 5-8w Striatal atrophy Astrogliosis	Impairment at cylinder test	Toxicity in distal regions (GP, SN) AAV vector-mediated RNAi prevents HD70 induced neuropathology
Hult 2011	AAV5 (Synapsin)	Htt171 (853) 18/79Q	Mice	Hypothalamus	Intranuclear Htt inclusions 6w No gliosis	↓motor activity 12-18w	Hyperphagia/Obesity 4w Deletion of mutant Htt in BACHD hypothalamus prevents metabolic phenotype
De Almeida 2002	Lv (PGK/CMV)	Htt171 (853,1520) 19/44/66/82Q	Rats	Striatum	Htt aggregates 1w Ubiquitin aggregates 2w DARPP32 loss 4w Astrogliosis 12w	-	Neuropathology correlates w/CAG repeats length and inversely w/Htt protein length
Regulier 2003	Lv (TRE/PGK tTA1)	Htt853 (171, 548) 19/82Q	Rats	Striatum	DARPP32 loss 1m Nuclear+cytoplasmic inclusions 1m	-	Htt expression level 6x reversed w/doxycycline as neuropathology
Palfi 2007	Lv (PGK)	Htt171 19/82Q	Macaca fascicularis	Putamen	Intranuclear+neuropil Htt aggregates 9w NeuN loss close to injection Astrogliosis	↑dyskinesia in apomorphine test 3-12w over 30w Spontaneous dyskinesia 9-16w over 30w	-

AAV: adeno associated virus; CBA: chicken β -actin promoter; CMV: cytomegalovirus promoter; DARPP32: dopamine and cAMP-regulated phosphoprotein of 32 kDa; GP: globus pallidus; Htt: huntingtin; Lv: HIV1 derived lentivirus; NeuN: neuronal specific nuclear protein; NSE: neuron-specific enolase promoter; PGK: phosphoglycerate kinase 1 promoter; SN: substantia nigra TRE: tetracycline-regulated; VTA: ventral tegmental area