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Non-human primate models of Huntington's disease and their application in translational research --Manuscript Draft--

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“Non-human primate models of Huntington’s disease and their application in translational research”

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Abstract

Huntington's disease (HD) is a monogenic, autosomal dominant inherited fatal disease that affects 1 in 10000 people worldwide. Given its unique genetic characteristics, HD would appear as one of the most straightforward neurodegenerative diseases to replicate in animal models. Indeed, mutations in the Htt gene have been used to generate a variety of animal models that displayed differential pathologies and that have significantly increased our understanding of the pathological mechanisms of HD. However, decades of efforts have also shown the complexity of recapitulating the human condition in other species. Here we will describe the three different types of models that have been generated in non-human primate species, stating their advantages and limitations and will attempt to give a critical perspective of their translational value to test the efficacy of novel therapeutic strategies. Obtaining construct, phenotypic and predictive validity has proven to be challenging in most animal models of human diseases. In HD in particular, it is hard to assess the predictive validity of a new therapeutic strategy when no effective "benchmark" treatment is available in the clinic. In this light, only face and construct validity will be discussed.

Keywords

Huntington's disease; animal models; non-human primates; preclinical efficacy; translational research

HD characteristics: what do we have to model ?

Huntington's disease is characterized, at the genetic level, by an expansion in the CAG triplet repeat in exon 1 of the huntingtin (HTT) gene. Such mutation results in an abnormally long (>36 residues) polyglutamine (polyQ) tract in the HTT protein and a relationship between the length of the tract and disease onset has been established (Duyao, Ambrose et al. 1993). At the functional level, the accumulation of the mutated HTT protein triggers its abnormal processing and impeded clearance, progressively leading to cellular toxicity and neurodegeneration (1993). In particular, the mutation induces a poorly described toxic gain of function within the cell (Tobin and Signer 2000, Shao, Welch et al. 2008) related in part to gene transcription, vesicle transport, and RNA trafficking (Cattaneo, Zuccato et al. 2005).

The pathological hallmark of the disease is the ubiquitous presence of ubiquitinated intracellular HTT aggregates in the nucleus and neuropile of neurons or in the cytoplasm of non-neuronal cells. Aggregates are mainly detected in the striatum, cortex and pallidum, hypothalamus (as well as in many brain regions inter-connected to the caudate-putamen complex such as the substantia nigra pars compacta, the thalamus) followed by neuronal loss and neuroinflammation of the striatum (Vonsattel, Myers et al. 1985). A characteristic neuropathological feature of HD is the "severe" cell death affecting mostly GABAergic medium sized spiny neurons (MSNs) in the striatum that gradually leads to atrophy of the striatum and interconnected structures such as the cerebral cortex (Ferrante, Kowall et al. 1985, Ferrante, Beal et al. 1987, Kowall, Quigley et al. 1993, Cicchetti, Prensa et al. 2000).

At the phenotypic level, a triad of motor, cognitive and psychiatric symptoms is observed and often accompanied by progressive muscle wasting and weight loss at late stages of the disease (Walker 2007). These symptoms evolve with time as the disease advances: the cognitive dysfunction appears first and includes executive function abnormalities like cognitive inflexibility, memory retrieval and procedural memory decline, visuo-spatial, attentional, concentration and motor planning deficits (Cummings 1995, Lawrence, Sahakian et al. 1996). At the psychiatric level,

depression, apathy and obsessive-compulsive behaviors are most common (Wang and Qin 2006). Psychiatric abnormalities such as anxiety, irritability, impulsivity, compulsivity, aggression, apathy and depression are commonly displayed by HD patients (Duff, Paulsen et al. 2007, Marshall, White et al. 2007). At the motor level, an initial presence of a hyperkinetic syndrome accompanied by choreiform movements is observed in 90% of patients and is later replaced by a triad dystonia, rigidity and bradykinesia (Young, Shoulson et al. 1986, Huntington 2003).

As for all animal models, the human condition determines their validity. HD being a monogenic disease with dominant and highly penetrant characteristics makes it, in theory, an easy disease to model. However, the size of the gene, the unstable nature of the mutation, the variability in CAG repeat number, the highly variable clinical presentation for a given CAG repeat number, as well as the variety of functions attributed to this ubiquitous protein have rendered pertinent animal models considerably complicated to obtain. A large spectrum of models have been generated in different species, from fruit flies to rodents to non-human primates, and all of them have been useful in increasing our understanding of the disease (reviewed in (Ramaswamy, McBride et al. 2007, Pouladi, Morton et al. 2013)). This review will concentrate only on non-human primates and the application of the different models available to the evaluation of existing or future therapeutic strategies.

Existing NHP models of HD: what have we managed to model?

Since HD has never been naturally observed in animals, the option of an ideal model, with face, construct and predictive validity, can be ruled out. The starting point was to mimic the massive cell death and atrophy observed in the caudate-putamen complex of advanced HD patients. The first HD models in NHP were thus generated using intra-striatal injections of glutamate agonists (excitotoxins) to induce cell death. Similarities between striatal lesions observed in HD patients and those observed following intrastriatal administration of excitotoxins such as ibotenic acid, kainate or quinolinic acid

in macaques, marmosets and baboons unilaterally and/or bilaterally initially supported the hypothesis of a direct glutamate receptor mediated neurotoxicity in HD (Kanazawa, Sasaki et al. 1985, Kanazawa 1986, DiFiglia 1990, Hantraye, Riche et al. 1990, Schumacher, Hantraye et al. 1992, Ferrante, Kowall et al. 1993, Brownell, Hantraye et al. 1994, Burns, Pakzaban et al. 1995, Kendall, David et al. 2000, Roitberg, Emborg et al. 2002). The use of quinolinic acid was later privileged over the other two because it is a naturally occurring product in the kynurenine pathway in the brain that was found to be abnormally activated in the striatum of HD patients (Schwarcz, Tamminga et al. 1988, Campbell, Charych et al. 2014) and because it could reproduce the typical pattern of striatal degeneration observed in HD i.e. preferential loss of GABAergic MSNs with relative sparing of other cell types such as the large cholinergic interneurons and the somatostatinergic interneurons as well as presynaptic striatal afferences such as the nigrostriatal dopaminergic pathway or the corticostriatal fibers (Beal, Kowall et al. 1986, Ferrante, Kowall et al. 1993, Gardian and Vecsei 2004). A phenotypical characterization of such excitotoxic models has shown spontaneous deficits in reaching tasks in some models (Kendall, David et al. 2000) but not others (Roitberg, Emborg et al. 2002); and evoked abnormal movements and hyperkinesia under apomorphine/dopamine agonist administration (Kanazawa, Sasaki et al. 1985, Hantraye, Riche et al. 1990, Burns, Pakzaban et al. 1995, Kendall, David et al. 2000); although dystonia was seldom observed. In most studies, the caudate-putamen was lesioned unilaterally and, only one study so far reports on cognitive deficits in QA-lesioned NHPs resulting from a bilateral lesion of the caudate nucleus (Roitberg, Emborg et al. 2002). Using the object retrieval detour task (ORDT), researchers have shown that the number of successful trials was decreased and the number of perseverative errors (barrier hits) was increased, consistent with a fronto-striatal dysfunction. Our laboratory has recently characterized this same model using tactile screens to deliver a battery of tests specific to fronto-striatal function (conceptual set shifting task, intra-extra dimensional shifting task, delayed-matching to sample task) and non-specific cognitive tests assessing visual discrimination and memory. Our results clearly show that 6

months after QA lesion, macaques display significant deficits in all specific tasks and no deficits in non-specific cognitive tests (Figure 1) (Aron Badin et al., under review).

With the observation that under conditions of mild energy impairment, striatal cells may become vulnerable to physiological concentration of glutamate agonists (the indirect excitotoxicity hypothesis), new models could be proposed and tested initially in rats (reviewed in (Brouillet, Conde et al. 1999). Malonate and 3-nitropropionic acid (3NP) are both mitochondrial toxins that interfere with the Krebs cycle, causing an energetic impairment and cell death in animals and humans (Ludolph, He et al. 1991). Remarkably, repeated intra-muscular administration of 3NP to several primate species results in selective degeneration of GABAergic MSNs of the caudate-putamen complex, usually starting in the dorsolateral aspect of the posterior putamen and dorsal part of the head of the caudate nucleus. These lesions not only trigger dystonia, dyskinesias of the face and the limbs, hyperactivity and chorea after apomorphine administration but also (depending on the intoxication regime used) can result in a persistent hyperkinetic syndrome (spontaneous dyskinesias and dystonia) in the absence of any pharmacological challenge (Beal, Brouillet et al. 1993, Brouillet and Hantraye 1995, Brouillet, Hantraye et al. 1995, Palfi, Ferrante et al. 1996, Palfi, Leventhal et al. 2000). A single study conducted in 3NP-lesioned baboons has shown cognitive abnormalities and perseverative behavior using the ORDT task (Palfi, Ferrante et al. 1996). Since ATP depletion and energy failure are still the underlying causative hypothesis of HD, this model recapitulates one important aspect of the disease and its functional consequences in terms of both motor and cognitive behavior but also metabolic abnormalities measured by MRI spectroscopy (Dautry, Conde et al. 1999, Dautry, Vaufrey et al. 2000). Post-mortem characterization of the lesions clearly shows cell death triggered both by apoptosis and necrosis, a loss of DARPP32 staining and overall striatal atrophy, all cardinal features of HD.

One of the caveats of direct injection of excitotoxins to the brain is that it is hard to control the extent of the lesion in other structures connected to the striatum, like cortical areas, that may be affected by the injection procedure and that are relevant to the pathology in patients. Moreover, the

targeted striatal territories will define the phenotype and the pathology and the reproducibility of excitotoxin injections can vary with stereotactic placement protocols (Burns, Pakzaban et al. 1995). This may be seen as an advantage since the striatal territories to be targeted can be defined a priori, but it is also a disadvantage regarding the disease since it is harder to mimic the gradients (dorso-ventral and rostro-caudal) of degeneration observed in patients. In contrast, systemic 3NP administration has been shown to produce bilateral lesions of the caudate-putamen complex starting in the dorsolateral aspects of both caudate and putamen but evolving with time according to a dorso-ventral gradient of DARPP32-positive immunoreactivity (reviewed in (Brouillet, Conde et al. 1999)). Interestingly, the pace of cell death differs between these two modalities: it is acute in intraparenchymal delivery and chronic in the systemic delivery. Neither, however, it reproduces the time-course of pathology observed in HD patients and more importantly, it has been argued that necrosis is the main mechanism of cell death compared to apoptosis. CAG-repeat dependent disease progression and severity of disease phenotype cannot be mimicked by toxins either. As for normal aging, in practical terms, it is not feasible to age animals long enough before the inoculation of toxins to generate a model gradually spanning over several decades.

Finally, the appearance of cognitive deficits before motor deficits is also hard to model when using excitotoxins as the cell toxicity produced by these glutamate agonists is evolving from neuronal dysfunction to actual neuronal death over few days (compared to several years in patients). In addition, whereas psychiatric alterations may be associated with excitotoxic lesions of the limbic parts of the striatum, such alterations are unfortunately intrinsically hard to evaluate in NHP models of neurodegenerative diseases and therefore have not been even studied so far.

The main features of the disease in HD patients are illustrated in Table 1.

Level	HD patients	NHP models	Species	Reference in NHP
Genetic	CAG repeats	CAG repeats	Macaca mulatta	Yang 2008; Chan 2014, 2015
Cellular	Apoptosis; Autophagy	Apoptosis; Necrosis	Papio anubis	Brouillet 1995, 1999 Brouillet 1995; Palfi, 1996

Functional	Cognitive symptoms	Cognitive symptoms	Cebus apella Papio anubis	Roitberg, 2002 Palfi, 1996
	Motor symptoms	Motor symptoms	Callithrix jacchus; Cebus apella; Macaca mulatta; Macaca fascicularis; Papio papio; Papio anubis	Kanazawa, 1986; Hantraye, 1989, 1990; Schumacher, 1992; Brownell, 1994; Storey, 1994; Burns, 1995; Palfi, 1996, 2000; Kendall, 2000; Roitberg, 2002; Aron Badin, under review
	Psychiatric symptoms	Not assessed	N/A	N/A
Neuro-chemical	GABA, Substance P, AChE	GABA, Substance P, AChE	Macaca mulatta	Ferrante, 1993
Neuro-pathological		Atrophy Aggregates	Macaca mulatta & Macaca fascicularis Papio papio & Papio anubis	Kanazawa, 1986, 1990; Ferrante, 1993; Palfi, 2000 Hantraye, 1989, 1990, 1992

Since the discovery of the mutation in Htt gene (1993) that underlies the onset of the disease in humans, many transgenic and knock-in models were generated in rodents (reviewed in (Pouladi, Morton et al. 2013). In later years, transgenesis has expanded to larger species like pig and sheep (Jacobsen, Bawden et al. 2010, Yang, Wang et al. 2010, Baxa, Hruska-Plochan et al. 2013, Morton and Howland 2013, Reid, Patassini et al. 2013, Handley, Reid et al. 2016) . Notably, incredible efforts were put into the engineering and the characterization of a transgenic HD model in rhesus monkeys (Wang, Tydlacka et al. 2008, Yang, Cheng et al. 2008, Chan and Yang 2009). The first lot of 6 animals presented a copy number-dependent and CAG-dependent severity in symptoms: one animal died before the term was completed, 2 animals died 1 day after birth, 1 animal died at 1 month of age and the follow-up of the fifth animal stops at 6 months after birth. The sixth animal and only long-survivor (2 year follow-up) showed some dystonia and chorea at early stages and a mild worsening of clinical scores and cognition (Chan, Xu et al. 2014). A second generation of animals was obtained expressing exons 1-10 of the Htt protein and a CAG repeat length of 67-72. The only behavioral

deficits observed after 16 months relate to motor execution and latency more than cognition since no significant impairment was found in the performance of ORDT or visuospatial orientation tasks (Chan, Jiang et al. 2015). A four year anatomical MRI follow up showed no significant reduction in caudate or putamen brain volume in transgenic NHPs compared to age-matched controls and overt tissue loss was only observed in 1 NHP. Moreover, a reduction in n-acetyl-aspartate (NAA) was detected by MR spectroscopy in transgenic monkeys at 48 months suggesting neuronal loss of function or actual neuronal death. From a neuropathological point of view, the second generation of transgenic HD NHPs seems to present a less severe pathology than the first generation of primates at 5 years of age. While NeuN counts do show neuronal loss in the caudate and putamen (to different extents in the 2 generations), EM48 positive intracellular aggregates are only obvious in the first generation of NHPs in the caudate and putamen and mutant Htt measurements by qPCR further confirm these post-mortem findings (Chan, Jiang et al. 2015). Although this HD model remains the closest to the human condition in terms of construct validity, as for transgenic mouse models, the copy number (human + NHP Htt), the insertion site(s) in the host genome, and the promoter used to drive expression can be an issue when modeling the human disease. The variability in the face validity (phenotypic presentation) and the time required to obtain a group of adult subjects at the same disease stage makes it difficult to envisage the use of this model for routine pre-clinical efficacy studies. In this light, viral overexpression of mutant Htt in specific cell types and defined areas of the brain could represent a viable alternative to study complex motor and cognitive behaviors after a shorter generation time. A lentiviral vector overexpressing the first exon of the huntingtin protein gene with either a171Htt-19Q (wild type) or a 171Htt-82Q (mutated) construct has been delivered unilaterally and bilaterally into the sensorimotor portion of the putamen and/or caudate of adult macaques. These studies have shown the presence of ubiquitinated Htt inclusions in neurites and cell bodies which triggered a loss of NeuN staining and an increase in astroglial reactivity (Figure 2) (Palfi, Brouillet et al. 2007). These pathological changes were accompanied by spontaneous motor abnormalities following bilateral viral vector delivery and apomorphine-induced chorea after

unilateral viral vector delivery. Recently, AAV vectors bearing similar constructs have been injected into the caudate and putamen of adult macaques in order to study the impact of aggregate accumulation in the striatum on both cognitive and motor behavior. An initial pilot study has shown good biodistribution of the viral particles leading to DARPP32 loss in the infected striatal regions and widespread EM48-positive aggregates at 3 months post-viral delivery (Aron Badin R, Callander G et al. 2013). A longitudinal study is ongoing to characterize the functional deficits and the resulting pathology at 18 months following viral vector delivery.

Relevance of modelling human disease in NHP: general considerations of translational research

It has been long argued that NHPs are essential in neuroscience research due to their phylogenetic proximity to humans and the million years of divergence with rodents (Phillips, Bales et al. 2014). Although this remains a fact, it is important to understand that the evolution of the brain, its use and the patterning arising from experience, have had functional consequences that translate into anatomical features and vice versa. Examples of this are the expansion of cortical layers, the specification of the pre-frontal cortex, the increase in surface in gyriform brains, the white matter connectivity and the higher density of neurons per structure (Semendeferi, Lu et al. 2002, Roth and Dicke 2005, Insausti 2013, Ventura-Antunes, Mota et al. 2013). Functionally, the sensory specificities of primates compared to rodents, like color vision, the use of hands and opposable thumbs, the tactile and visual reliance rather than olfactory, dictate the sub-regional organization of the brain. Thus, the use of vibrissae in rodents versus the use of hands in NHPs is reflected in the organization of the primary somatosensory cortex (Seelke, Dooley et al. 2012). The behavioral repertoire of the species is a consequence of brain anatomy and connectivity and this can be important when assessing efficacy and safety of certain treatments like gene- or cell-based therapies. In order to rule out or record adverse events in pre-clinical studies, as for example spontaneous limb choreic or dyskinesic movements, those behaviors must be present in the species used and this is not always

the case in rodents (Brouillet, Conde et al. 1999). For example, excitotoxic striatal lesions or striatal lesions induced by chronic systemic 3NP administration do not induce evoked nor spontaneous abnormal movements in rats whereas they do evoke a variety of choreatic and dystonic movements in NHP (Hantraye, Palfi et al. 2000), suggesting that some types of dyskinesia, components of HD symptomatology, can only be expressed in the NHP behavioral repertoire. Similarly, the outcome measures used to assess the efficacy of a given therapeutic strategy need to be as close as possible to those used in clinical trials as primary or secondary end-points in order to increase the predictivity of the pre-clinical results obtained (Aron Badin and Hantraye 2012, Aron Badin, Vadori et al. 2015). In this respect, behavioral and imaging outcomes are preferable. Imaging techniques that are non-invasive and have a high translation value like magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS) and, in particular, positron emission tomography (PET) or single photon emission computed tomography (SPECT) imaging can be affected by the size of the structure under study due to the spatial resolution of available scanners (Wolfe, 2009). This is particularly true for the brain, where a sub-anatomical level precision in certain types of imaging techniques may benefit from a larger organ size. Moreover, the overall size of the brain is another important consideration for scaling-up issues, both in the generation of models and in the assessment of therapeutic strategies. As an example, the distance between functionally connected structures and the transport of therapeutic agents across axons of different lengths in rodent and primate species can affect the outcome of a model or the design of a clinical trial. Finally, the availability of peripheral fluids like blood and CSF has proven invaluable for the longitudinal follow-up in the search for biomarkers of disease progression and/or modification after therapy. The longitudinal access to such fluids can help inform researchers about a potential immunization after gene therapy, the production of antibodies and even the rejection of cell therapy products. It is important that the species used displays the potential to react to a given therapeutic product or cellular, viral, or chemical origin, in the same way as humans and some toxicological studies have highlighted differences between primate and rodent species in this regard (Haley 2003).

Uses of models to test therapies

Although it is impossible to study the timecourse and disease progression in models generated acutely or sub-chronically by toxin administration, these models have been extensively used to test therapeutic strategies aiming at protecting the brain from loss of function and cell death or restoring the lost functions. In particular, the evaluation of the survival, implantation, differentiation and functional impact of cell-based therapies has greatly benefited from these models of HD. Numerous cell transplantation strategies have been attempted in baboons, macaques, and marmosets to rescue the functional and neuropathological consequences of ibotenic acid, quinolinic acid or 3NP toxic lesions (Simmons, Pastakia et al. 1986, Isacson, Riche et al. 1989, Isacson, Hantraye et al. 1990, Denys, Leroy-Willig et al. 1992, Hantraye, Leroy-Willig et al. 1992, Schumacher, Hantraye et al. 1992, Simmons, Helm et al. 1994, Palfi, Ferrante et al. 1996, Palfi, Conde et al. 1998, Kendall, David et al. 2000, Emerich, Thanos et al. 2006) . These studies have highlighted the importance of the integrity of the cortico-striato-pallidal circuitry, the ability to compensate its disruption with cellular grafts of different origin (from fetal cells to choroid plexus) and species and the positive functional impact on motor and cognitive behavior. An ongoing study is evaluating the effect of stem cells, and in particular human induced pluripotent (iPS)-derived medium-sized spiny neurons, in a primate model of HD looking at survival, functional integration and immunogenicity of striatal grafts (<http://www.repair-hd.eu/>). The objective of this study is to generate a clinical grade bank of differentiated human cells with proven safety and efficacy and the design of a clinical trial protocol for stem cell transplantation of HD patients.

The reduction in the levels of brain-derived neurotrophic factor (BDNF) has been implicated in the progression of neuronal death in HD (reviewed in (Zuccato and Cattaneo 2007)). Such decrease has also been observed in a primate model of HD (3NP-treated macaques) but can be reversed after the administration of cysteamine (Borrell-Pages, Canals et al. 2006). Several strategies that deliver

trophic factors to the brain have been tested in non-human primates to prevent neuronal death and to slow down the progression of the disease. A proof of concept of the beneficial effect of this approach came by implanting encapsulated genetically-engineered cells overexpressing nerve growth factor (NGF) directly close to the lesion site (Emerich, Winn et al. 1994, Kordower, Winn et al. 1994). A few years later, ciliary neurotrophic factor (CNTF) was delivered to the primate brain before or after lesion using engineered encapsulated cells or viral vectors (Emerich, Winn et al. 1997, Mittoux, Joseph et al. 2000). These pivotal studies helped establish the therapeutic benefit of neurotrophic factors and helped elucidate the design of clinical trials for both neuroprotection/disease modification and neurorestoration (Bachoud-Levi, Deglon et al. 2000, Bloch, Bachoud-Levi et al. 2004, Moirano and Emborg 2006, Wolfe 2009, Ramaswamy and Kordower 2012, Ruozi, Belletti et al. 2012, Emborg, Hurley et al. 2014).

A different disease modifying strategy relies on the reduction in mutant Htt load to prevent or reduce the downstream pathological effects. Several gene silencing approaches have been tested in rodents using short nucleic acid sequences of DNA or RNA to bind to specific mRNA sequences and target them for degradation to prevent their translation into mutant proteins. In particular, short hairpin RNA (shRNA), small interfering RNA (siRNA), and short antisense oligonucleotides (ASOs) have shown efficacy and clinical trials are ongoing (Pfister, Kennington et al. 2009, Godinho, Malhotra et al. 2015, Keiser, Kordasiewicz et al. 2016). Because this type of treatment requires more than a single administration, and because the blood-brain-barrier renders penetrance into the brain and target engagement very challenging after systemic delivery, direct intracerebral (parenchymal or ventricular) routes have been chosen. In the particular case of shRNA that needs to be produced locally, viruses of different origins have been engineered to transduce the target cell population in the brain (Ruiz and Deglon 2012). The availability of viral vectors that target non-dividing cell populations in an efficient and selective manner is also determinant in the success of these therapeutic strategies and a recent study addresses the biodistribution of a reporter gene in the cortico-striatal territories of adult primates using AAV1 and AAV2 capsids (Hadaczek, Eberling et al.

2010). It should be mentioned that the presence of pre-existing neutralizing antibodies or the generation of these after viral delivery, has been shown to compromise the therapeutic efficacy. Pre-screening for naturally existing antibodies can be easily performed in patients and non-human primates and can ensure a long-term stable expression of the transgene of interest. Another important point is that while allele-specific elimination of solely the mutant Htt seems to be the most desirable therapeutic strategy, it is also, at this stage, the most challenging one from a technical point of view (Drouet, Perrin et al. 2009, Lombardi, Jaspers et al. 2009, Ostergaard, Southwell et al. 2013). On the other hand, lowering simultaneously normal and mutant Htt protein is technically easier but could potentially have undesirable adverse effects that need to be assessed in vivo. Many studies have looked at these issues in rodent species but two publications in intact NHP have also reported on the safety and distribution of ASO and siRNA silencing strategies. One study has used AAV vectors to deliver siRNA to the sensorimotor putamen of adult macaques. Results at 6 weeks post-viral vector delivery show no alterations in body weight, overall clinical score, locomotor activity or fine motor skills using the lifesaver task. Post-mortem analysis showed that aside from a mild mechanical disturbance of the tissue around injection sites, there was no neuronal loss (NeuN, DARPP32), no overall increase in gliosis (GFAP, Iba1) or in humoral response (CD4, CD8) in the brain at this timepoint. Finally, the viral construct used transfected approximately 30% of the putamen including medium-sized spiny neurons, aspiny interneurons, and astrocytes and yielded a 45% reduction in Htt in the absence of toxicity (McBride, Pitzer et al. 2011). Similar results were obtained by another group at 6 months following intrastriatal delivery of an AAV2 vector overexpressing a shRNA that caused a 30% reduction in Htt mRNA and a 45% reduction in Htt protein in the absence of neurodegeneration or inflammation in the brain (Grondin, Kaytor et al. 2012). The same group studied the effect of a continuous delivery of a naked siRNA directly into the putamen of adult primates for 7 or 28 days and found this approach was well tolerated, achieving a good biodistribution of the siHtt that was dose- and time-dependent, as well as a widespread significant reduction of Htt levels in the absence of off-target toxicity (Stiles, Zhang et al. 2012). These findings

were further confirmed by analyzing the duration of the effect of intraputaminial infusion of the siHtt for 1 to 3 days and quantifying the reduction in Htt levels at 0, 10 and 24 days after treatment instead of infusing the siRNA continuously in adult NHPs (Grondin, Ge et al. 2015). Finally, a 21-day continuous intrathecal delivery of ASOs showed that Htt protein levels were significantly reduced by 47% in cortical regions and a non-significant 25% reduction was seen in the caudate nucleus immediately after treatment. The cortical areas showed persistent reduction of Htt at 4 weeks post-treatment and a recovery of Htt levels was observed by 8 weeks following treatment (Kordasiewicz, Stanek et al. 2012). Overall, these results suggest that partially silencing total Htt is safe and well tolerated in NHPs, and that the levels of silencing achieved are comparable to those proved to be beneficial in rodent models of HD. Arguably, the availability of genetic or transgenic models of HD in NHPs would allow to test the safety, the distribution, the off-target effects and the efficacy of any silencing strategy in the same cohort.

Future perspectives: models and therapies

The main challenges to be overcome in the next few years involve target engagement, specific efficacy and toxicity as well as the lack of a reliable biomarker of disease progression. For agents being delivered to the brain, viruses, nucleic acids or drugs, the penetration of the therapeutic products into the brain (crossing the blood-brain-barrier (BBB), avoiding degradation outside and inside the brain) will depend on the nature of the molecule or vector and the delivery route. A systemic, intrathecal or intraparenchymal delivery and its frequency (single shot or repeated administration over time) will influence the reaction of the immune system to such foreign agents, whether it is viruses, nucleic acids or transplanted stem cells.

The crossing of the BBB has received increasing attention in the last decade by trying to improve the systemic delivery of antibodies, drugs and nucleic acids. As mentioned before, degradation of the compounds and penetration into deep structures in the brain as well as targeted cellular/regional

specificity is a major issue. In this regard, evidence on the feasibility and safety of focal and reversible BBB opening using ultrasounds is accumulating in NHPs and technological advances could accelerate the application of this delivery approach thus increasing the efficacy of the therapeutic compounds delivered (Downs, Buch et al. 2015).

For strategies aiming at lowering HTT mRNA and protein, the surcharge of the cellular machinery over decades could also lead to cellular toxicity and death, and consequently to the activation of the immune system. Moreover, the fact that these strategies are not specific for mutant HTT opens the possibility of “off-target” effects on non-pertinent cells and on other proteins in the genome bearing CAG repeats, which could lead to further function and immunological complications.

The use of viral and biodegradable non-viral delivery methods needs further investigation to avoid toxicity and increase specificity. The possibility to perform direct genome editing to repair or replace the mutant DNA sequence using sophisticated endonuclease systems (transcription activator-like effector nucleases, zinc-finger nucleases, meganucleases or clustered regulatory interspaced short palindromic repeat systems) has to be further documented in large animal models to assess any potential detrimental effects over time.

Ongoing clinical trials will provide valuable information on strategies designed to restore the dysfunction in the basal ganglia by targeting protein accumulation, energy impairments and physiology. Several therapeutic approaches aim at reducing cellular excitotoxicity by glutamate receptor inhibition and GDNF increase (riluzole, memantine), conferring neuroprotection against caspase activity (minocycline and caspase-6 inhibitors), activating the ubiquitin-proteasome system or the autophagy-lysosomal pathway to increase HTT clearance, and protecting mitochondrial energetic activity (creatine, coenzyme Q10, cysteamine) (Kumar, Kumar Singh et al. 2015). Since most of these therapeutic strategies have been tested only in mice, their outcome will in turn inform researchers of the predictability of these models when scaling up and may point to the need of using NHPs species to substantiate and refine future clinical trials. An interesting alternative to targeting

these downstream pathways resulting from Htt accumulation, is to use intrabodies (intracellular antibodies) or Q-rich prion-like proteins to interfere with the PolyQ expansion domain, change its physical properties and prevent it from aggregating into large fibrils that sequester other proteins thus reducing the toxic gain of function of mutated Htt (Ripaudo, Chumakova et al. 2014). Although the role of astrocytes is well established in neurodegenerative diseases like HD, there is mixed evidence in the literature for their beneficial and detrimental effects (Faideau, Kim et al. 2010, Crotti and Glass 2015). A recent complex mouse study has attempted to shed light on the potential pathological role of glial progenitors derived from HD patients and found significant motor and electrophysiological disturbances in transplanted wild type animals. Moreover, engrafting wild type fetal glial cells increased overall survival and partially rescued both the behavioral phenotype and neuronal physiology in transgenic HD mice (Benraiss, Wang et al. 2016). This evidence and previously published data open the way for the further investigation of resident glial manipulation or transplantation to slow down disease progression and correct symptoms in HD patients (Escartin and Bonvento 2008).

Finally, a significant reduction in CYP46A1, a rate-limiting, neuron-specific cholesterol enzyme, has been detected in the post-mortem brain of HD patients and in transgenic mouse models of the disease where the phenotype can be restored by gene therapy with a CYP46A1-overexpressing viral vector. Conversely, knocking out this enzyme in the striatum of wild type mice using an AAV viral vector overexpressing a specific shRNA has been shown to generate a phenotypic model of HD (Boussicault, Alves et al. 2016). These data further support the established role of cholesterol as a neuroprotective agent, and a pre-clinical study is ongoing in intact and HD NHPs to scale-up this therapeutic strategy to the clinic (Cartier, personal communication).

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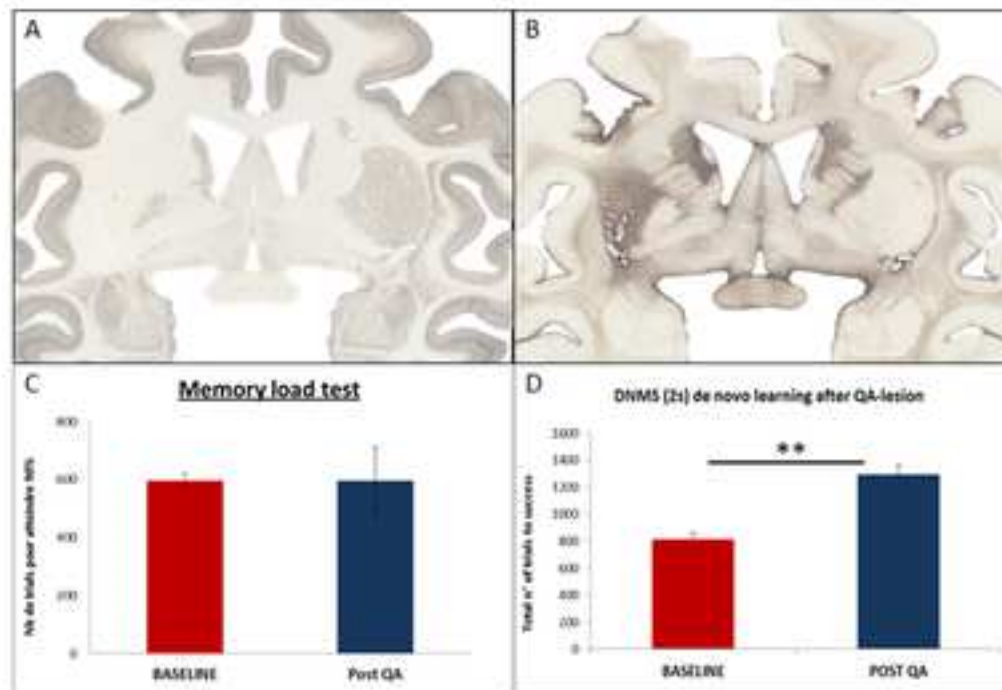


Figure 1. Effect of an excitotoxic QA lesion bilaterally in the caudate and unilaterally in the left putamen 6 months after surgery in an adult macaque. Representative coronal sections showing the loss of neurons (NeuN, panel A) and the increase in reactive astrocytes (GFAP, panel B) in the right and left caudate and in the left putamen as well as the absence of lesion in the right putamen. These QA lesions result in specific behavioral deficits illustrated by two cognitive tests delivered on touch screens at baseline and 6 months after surgery. We observed no significant difference in performance of a non-striatal memory test (panel C) and a significant difference ($p < 0.001$) in a specific executive task, delayed no-matching to sample (DNMS). No NeuN loss or GFAP staining were observed in the hippocampal formation (data not shown).

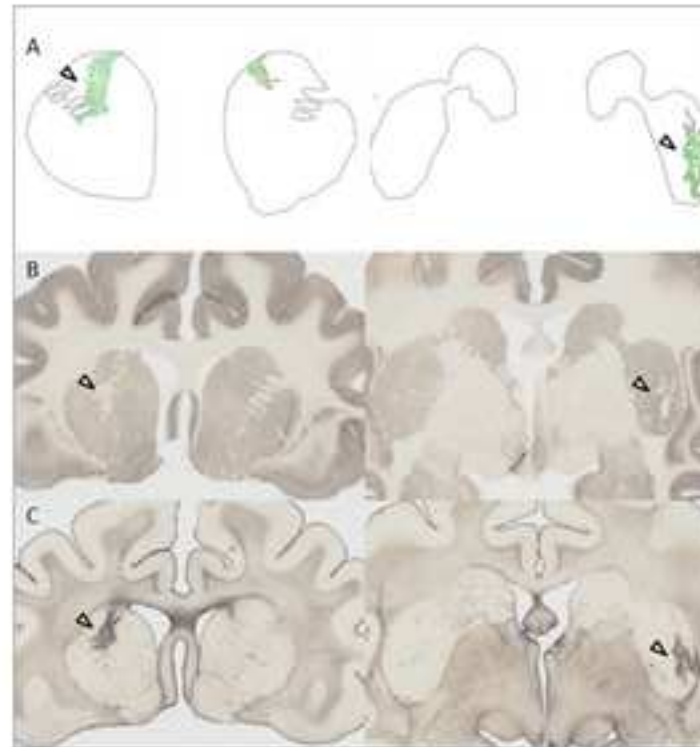


Figure 2. Post-mortem analysis showing immunohistochemical results at 15 months post-injection of a lentiviral vector overexpressing the mutated huntingtin protein in the caudate (left panel) and putamen (right panel) of an adult macaque. Representative coronal sections showing the cartography of the localization of mutated huntingtin aggregates (EM48, A) on a single antero-posterior level, accompanied by the loss of neurons (NeuN, B) and an increase in reactive astrocytes (GFAP, C) in the same areas.

Level	HD patients	NHP models	Species	Reference in NHP
Genetic	CAG repeats	CAG repeats	Macaca mulatta	Yang 2008; Chan 2014, 2015
Cellular	Apoptosis; Autophagy	Apoptosis; Necrosis	Papio anubis	Brouillet 1995, 1999; Brouillet 1995; Palfi, 1996
Functional	Cognitive symptoms	Cognitive symptoms	Cebus apella Papio anubis	Roitberg, 2002; Palfi, 1996
	Motor symptoms	Motor symptoms	Callithrix jacchus; Cebus apella; Macaca mulatta; Macaca fascicularis; Papio papio; Papio anubis	Kanazawa, 1986; Hantraye, 1989, 1990; Schumacher, 1992; Brownell, 1994; Storey, 1994; Burns, 1995; Palfi, 1996, 2000; Kendall, 2000; Roitberg, 2002; Aron Badin, under review
	Psychiatric symptoms	Not assessed	N/A	N/A
Neuro- chemical	GABA, Substance P, AChE	GABA, Substance P, AChE	Macaca mulatta	Ferrante, 1993
Neuro- pathological		Atrophy Aggregates	Macaca mulatta & Macaca fascicularis Papio papio & Papio anubis	Kanazawa, 1986, 1990; Ferrante, 1993; Palfi, 2000; Hantraye, 1989, 1990, 1992

Table 1. Summary of the characteristics of Huntington's disease observed in patients and recapitulated in non-human primates.