

## Huntington's disease: mechanisms of pathogenesis and therapeutic strategies

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### Abstract

Huntington's disease is a late onset neurodegenerative disease caused by a CAG trinucleotide repeat in the gene encoding the huntingtin protein. Despite its well defined genetic origin, the molecular and cellular mechanisms underlying the disease are unclear and complex. Here, we review some of the currently known functions of the wild type huntingtin protein and discuss the deleterious effects that arise from the expansion of the CAG repeats, which are translated into an abnormally long polyglutamine tract. Finally, we will outline some of the therapeutic strategies that are currently being pursued to slow down the disease.

### 1. Introduction to Huntington's disease: genetics and pathology

Huntington's disease (HD) is an autosomal dominant condition characterized by movement disorders and cognitive decline. Typically, the motor defects include chorea and loss of coordination. Psychiatric symptoms, such as depression, psychosis and obsessive-compulsive disorder, are also common in HD and are particularly distressing for patients (Rosenblatt 2007). The prevalence of the mutation is 4–10 cases per 100,000 in populations of Western European origin.

HD is characterized by a general shrinkage of the brain and degeneration of the striatum (caudate nucleus and putamen), with specific loss of efferent medium spiny neurons (MSNs) (Reiner et al. 1988). Although the striatum appears to be the most affected region of the brain, a regionally specific thinning of the cortical ribbon was found in patients with HD (Rosas et al. 2002). Such loss of cortical mass is an early event in the pathology of HD and proceeds from posterior to anterior cortical regions with disease progression. This regionally selective cortical degeneration may explain the heterogeneity of clinical expression in HD. Additional features are often present in HD patients, such as weight loss, skeletal-muscle wasting and cardiac failure (Aziz et al. 2008) (Arenas et al. 1998). Although generally less investigated than neurological signs, these additional signs might be due to the ubiquitous expression of mutant huntingtin (the toxic protein that causes HD).

HD is due to mutations in the *HTT* gene encoding huntingtin, a ubiquitously expressed protein of 350 kDa (1993). Huntingtin contains a polyglutamine tract encoded by uninterrupted CAG trinucleotide repeats in the first exon of *HTT*. Wild-type alleles contain up to 35 CAG repeats, whereas HD patients carry expansions of 36 or more repeats (Rubinsztein et al. 1996). Although complete penetrance of

HD is observed for CAG sizes of  $\geq 42$ , only a proportion of those with a CAG repeat length of 36-41 show signs or symptoms of HD within a normal life span (Rubinsztein et al. 1996)( Brinkman et al. 1997).

There is a strong inverse correlation between the number of CAG repeats and the age of onset of symptoms: larger CAG repeat expansions are generally associated with earlier ages of onset (Andrew et al. 1993). However, the CAG repeat number only partially explains 65-71% the variance in the age of onset, which also appears to be influenced by additional environmental and genetic factors, like modifier genes (Rosenblatt et al. 2001). Moreover, monozygotic twins have been reported to show different clinical symptoms, suggesting that epigenetic factors or tissue-specific variation in CAG repeats, due to somatic instability, may influence the disease (Georgiou et al. 1999).

HD is also characterized by the phenomenon of anticipation, where the age of onset tends to decrease in successive generations. This is due to the unstable nature of the CAG repeats that tend to increase in size, particularly when passed through the male germline (Trottier et al. 1994). While germline instability can explain the phenomenon of anticipation, somatic instability has been proposed as mechanism underlying the tissue specificity of the disease.

In order to study the pathophysiology of HD, several mouse models have been generated. For an exhaustive description of those models, refer to reviews: (Menalled and Chesselet 2002) (Lee et al. 2013).

## **2. Wild type huntingtin: structure and functions**

Huntingtin is a ~350 kDa protein containing the polyglutamine sequence at the NH<sub>2</sub> terminus and multiple consensus sequences called HEAT (huntingtin, elongation factor 3, protein phosphatase 2A, and TOR 1) repeats that are important for protein-protein interactions. HEAT motifs have a helix-turn-helix structure that is tightly packed to form a superhelix hydrophobic core that resists dissociation after proteolytic cleavage (Li et al. 2006). These motifs are often present in proteins involved in intracellular trafficking, such as clathrin adaptors and COP-I coatomer (Neuwald and Hirano 2000) and are possibly responsible for the scaffolding role of huntingtin in the formation of protein complexes (Takano and Gusella 2002).

Huntingtin is a cytoplasmic protein with partial nuclear localization. Recently, its NLS (nuclear localization sequence) has been described in the amino terminus of the protein (Desmond et al. 2012). It spans between amino acids 174 and 207 and interacts with karyopherin  $\beta$ 2, a protein that mediates nuclear import of proteins. This NLS comprises three consensus components: a basic charged sequence, a downstream conserved arginine, and a proline-tyrosine (PY) sequence.

Huntingtin also contains a NES (nuclear export sequence) in the carboxy-terminus (Xia et al. 2003). Moreover the N-terminal sequence of huntingtin interacts with Tpr, a nuclear pore protein that is involved in nuclear export. Polyglutamine expansions decrease this interaction and increase the nuclear accumulation of huntingtin (Cornett et al. 2005).

Huntingtin is widely expressed in humans and rodents, with highest levels in the neurons of the CNS, where it appears to localize predominantly in the cytoplasm and be associated to vesicle membranes (DiFiglia et al. 1995). In particular, huntingtin is enriched in scattered striatal large neurons and in all corticostriatal neurons (Fusco et al. 1999).

## **Physiological functions of huntingtin**

Since the discovery of *HTT* as gene responsible for HD, efforts have been made to elucidate the function of wild type huntingtin and several roles have been described so far. Here we will summarize the most studied ones.

### ***Huntingtin is necessary for embryonic development***

Huntingtin is required for early embryonic development, as knockout mice show embryonic lethality around day 8.5, before the emergence of the nervous system (Nasir et al. 1995) (Zeitlin et al. 1995). Moreover, recent studies reveal that huntingtin plays a crucial role in neurogenesis. In fact, huntingtin was shown to be required for the maintenance of the lineage potential of primitive neuronal stem cells during the process of neural induction (Nguyen et al. 2013). Furthermore, huntingtin has a crucial role in neurulation controlling homotypic interactions between neuroepithelial cells (Lo Sardo et al. 2012). This function is executed by inhibiting both the activity of the metalloprotease ADAM10 and N-cadherin cleavage. This also occurred *in vivo*, as defects in neural tube morphogenesis that were observed in huntingtin knockdown zebrafish embryos could be rescued after treatment with GI254023X, an ADAM10 inhibitor.

### ***Huntingtin acts as a protein scaffold***

Wild-type huntingtin is a well-characterised scaffolding protein. It interacts with  $\beta$ -tubulin and binds to microtubules (Hoffner et al. 2002). It also interacts with the dynein/dynactin complex (Caviston et al., 2007), regulating several intracellular trafficking processes. Recently, huntingtin has been shown to localize to spindle poles during mitosis controlling spindle orientation in mouse neuronal cells (Godin et al. 2010). In the absence of huntingtin, dynein/dynactin and NuMA were dispersed around the spindles poles. Therefore, huntingtin possibly functions as a scaffold molecule that orchestrates the assembly of the dynein/dynactin complex.

### ***Huntingtin as a transcriptional regulator***

The nuclear localization confers huntingtin a role in transcriptional regulation (Kegel et al. 2002). Although numerous transcription factors are known to interact with mutant huntingtin, less is known about the interactions with the wild-type protein. A well-known target of huntingtin-mediated transcriptional regulation is the gene encoding BDNF (brain-derived neurotrophic factor) (Zuccato et al. 2003). In the cytoplasm, wild type huntingtin sequesters and inhibits the activity of REST/NRSF (repressor element-1 transcription factor/neuron restrictive silencer factor), a transcription factor that negatively regulates BDNF transcription. Recently, it has been shown that huntingtin interacts with methyl-CpG binding protein 2 (MeCP2) in mouse and cellular models of HD. This interaction may also modulate the huntingtin-mediated expression of BDNF (McFarland et al. 2014).

### ***Huntingtin in the synapse***

A new emerging role of huntingtin is in synaptic connectivity. Huntingtin is associated with synaptic vesicles in the presynaptic terminal (DiFiglia et al. 1995), as well as in the postsynaptic density (Marcora and Kennedy 2010), where it is associated with the scaffolding protein PSD95 (Sun et al.

2001). For many years, the role of huntingtin in this compartment was obscure. A recent study showed that huntingtin is required for a correct formation of cortical and striatal excitatory synapses (McKinstry et al. 2014). In particular, when huntingtin was silenced in developing mouse cortex, an increase in excitatory synapse formation in the cortex and striatum was observed at P21, followed by gliosis.

### **3. Mechanisms of pathogenesis in Huntington's disease**

Despite the well-known genetic origin of HD, the number and variety of molecular alterations reported in HD is broad and not completely understood. While it is known that toxicity in HD arises from a gain-of-function of the mutant protein, given that expression of an expanded polyglutamine is toxic itself, a contribution of a loss-of-function of the wild-type protein cannot be discarded because deletion or inactivation of wild-type huntingtin also leads to neurodegeneration (O'Kusky et al. 1999)(Dragatsis et al. 2000).

We will outline here some of the mechanisms of pathogenesis described to date and focus in particular on those which are related to potential targets for therapy.

#### ***Mutant huntingtin aggregation: is it protective or deleterious?***

The hallmark of HD, and common to other polyglutamine disorders, is the presence of aggregates in the brain. These were initially considered crucial in HD pathology. Similar to other polyglutamine containing proteins, mutant huntingtin aggregation proceeds by nucleated growth polymerization (Perutz and Windle 2001), leading to polyglutamine strands forming a beta-sheet held together by hydrogen bonds (Perutz et al. 1994) which results in an amyloid structure (Chen et al. 2002)(McGowan et al. 2000).

HD aggregates, initially found in the nucleus (DiFiglia et al. 1997)(Becher et al. 1998) and later also in the cytoplasm and neuronal processes in the brain of HD patients (Gutekunst et al. 1999), are composed mainly of the expanded mutant huntingtin but also of many other proteins including ubiquitin (Becher et al. 1998)(DiFiglia et al. 1997), proteasome subunits and chaperones (Cummings et al. 1998)(Warrick et al. 1999), transcription factors (Huang et al. 1998) (Steffan et al., 2000), or even the wild type form of huntingtin (Kazantsev et al. 1999)(Busch et al. 2003). Hence, the idea of a deleterious effect as a consequence of the loss of functional proteins sequestered into these aggregates was quite appealing. Another argument on behalf of their pathogenicity is that the number of polyglutamines correlates both with the rate of aggregation and with the onset of the disease (Becher et al. 1998) (Martindale et al. 1998) (Perutz and Windle 2001), which suggests a direct link between aggregation and cell toxicity (Hackam et al. 1998).

Contrary to this intuitive hypothesis, there are also arguments supporting the idea that aggregation does not correlate with toxicity, and that aggregates might be just coincidental or even protective in HD (Saudou et al. 1998), (Kim et al. 1999) and other polyglutamine disorders (Klement et al. 1998)(Cummings et al. 1999). Single living neuron studies inferred an inverse correlation between the presence of aggregates and cell death (Arrasate et al. 2004) and suggested a protective role for these inclusions by sequestering toxic soluble species. In this line, the toxicity of the different mutant huntingtin species is currently a matter of debate: monomeric huntingtin forms soluble oligomers that precede fibrils and inclusions (Poirier et al. 2002)(Mukai et al. 2005)(Legleiter et al.

2010) and many reports point at these oligomers as the toxic species (Takahashi et al. 2008)(Lajoie and Snapp 2010)(Lajoie and Snapp 2013). Understanding which are the genuine harmful species is crucial to design therapeutic strategies.

The aggregates in adult-onset HD are typically cytoplasmic, while those in juvenile-onset disease were proposed to be more frequent in the nucleus (DiFiglia et al. 1997)(Becher et al. 1998). A recent study has suggested that some aggregates that appear to be nuclear may indeed be perinuclear, i.e. cytoplasmic. These perinuclear aggregates have been proposed to be the toxic species which appear to cause cell death by abnormally activating cell cycle (Liu et al. 2014). This study may resolve some of the controversy about the roles of aggregates in HD, as it appeared that the truly nuclear aggregates were relatively benign, compared to the perinuclear aggregates, and that diffuse mutant huntingtin does not impact on cell death. However, the conclusions are still somewhat at odds with previous studies that suggested that the mutant huntingtin was most toxic in its non-aggregated state (Arrasate et al. 2004).

### ***Huntingtin is cleaved in toxic fragments***

Accumulation of pathogenic N-terminal fragments of huntingtin is characteristic in HD (Davies et al. 1997)(Kim et al. 1999)(Mende-Mueller et al. 2001). These fragments come from diverse origins, including proteolysis by caspases (Hermel et al. 2004) (Wellington et al. 1998)(Wellington et al. 2000) (Kim et al. 2001)(Wellington et al. 2002)(Graham et al. 2006), calpains (Gafni et al. 2004)(Bizat et al. 2003)(Kim et al. 2003) and other proteases. In addition, alternative mechanisms might contribute, such as the aberrant splicing of the first exon of huntingtin protein (Sathasivam et al. 2013)

Although both wild-type and expanded huntingtin get cleaved, the presence of mutant fragments correlate with increased toxicity, which might be due to their higher propensity to form nuclear versus cytoplasmic less toxic aggregates (Hackam et al. 1998)(Lunkes and Mandel 1998) (Kim et al. 1999)(Lunkes et al. 2002). Also, the nature of these fragments may vary between tissues, which might contribute to differences in cell susceptibility (Mende Mueller 2001)(Toneff et al. 2002)(Wellington et al. 2002). Hence, inhibiting the formation of these fragments has been pursued as a therapeutic strategy, which could be achieved also indirectly, for example by modifying the susceptibility of cleavage by phosphorylation of huntingtin by Cdk5 (Luo et al. 2005), or phosphorylation of a domain which impairs calpain cleavage (Schilling et al. 2006).

### ***Mutant huntingtin disrupts transcription***

Transcriptional dysregulation has long been considered as a major pathogenic mechanism in HD. DNA microarray studies have revealed that expression profiles of a number of genes are profoundly altered in HD (Luthi-Carter et al. 2000)(Luthi-Carter et al. 2002)(Sipione et al. 2002). The activation domains of many transcription factors are composed of glutamine-rich regions suggesting that they may interfere with expanded polyglutamines. Indeed, mutant huntingtin interacts with regulators of transcription such as p53, cAMP response element-binding (CREB) protein and CREB-binding protein (CBP), involved in cell proliferation and survival (Steffan et al., 2000)(Nucifora et al. 2001)(Sugars et al. 2004); PGC-1 $\alpha$ , which is necessary for energy metabolism (Cui et al. 2006)(Chaturvedi et al. 2010); Sp1 and its coactivator TAFII130, affecting transcription of genes such as D2 dopamine receptor (Dunah et al. 2002) (Zhai et al. 2005); and cystathionine  $\gamma$ -lyase (CSE), the biosynthetic enzyme for cysteine (Paul et al. 2014), among many others.

The increased susceptibility of the striatum in HD has been attributed to a reduction in the levels of the brain-derived neurotrophic factor (BDNF), a pro-survival factor produced cortically to promote survival of striatal neurons. Impairment in transcription (Zuccato et al. 2001) or in axonal transport of BDNF (Gauthier et al., 2004) or its receptor TrkB (Liot et al., 2013) are all mechanisms that have been proposed to contribute to this deficit. In addition, corticostriatal synaptic defects in mouse models have been recently attributed to defects in BDNF signalling, rather than reduce BDNF levels, through an impact on postsynaptic p75 neurotrophin receptor (Plotkin et al. 2014), which along with TrkB binds to BDNF and is also implicated in HD (Brito et al. 2013)(Simmons et al. 2013)(Jiang et al. 2013).

### ***Alterations in gene expression beyond transcription: epigenetics and non-coding RNAs***

Gene expression dysregulation in HD might also arise from variations in the epigenetic landscape, as well as in the regulation of non-coding RNAs. A first hint of deregulation of histone modification in HD came from the study of CBP, a transcriptional co-activator with histone acetyltransferase (HAC) functions. Expanded polyglutamines can bind to the HAC domain of CBP as well as other HACs, which disrupts their histone acetylation activity. Likewise, histone deacetylase (HDAC) inhibitors prevent neurodegeneration in cells, *Drosophila* or mouse models of HD (Steffan et al. 2001)(McCampbell et al. 2001)(Hockly et al. 2003)(Ferrante et al. 2003). More recently, genetic inhibition of HDAC4 has been shown to restore neurological dysfunction and extend life span in HD mouse models independently of its histone deacetylase function but due to reduced aggregate formation through decreased interaction between expanded polyglutamines and its glutamine-rich domain (Mielcarek et al. 2013).

In an effort to determine chromatin structural modifications in the genes downregulated in HD, a genome-wide approach identified a specific H3K4me3 pattern, a mark of active chromatin and transcription initiation, which correlated with transcriptional dysregulation in the R6/2 HD mouse and human HD brain (Vashishtha et al. 2013). Along similar lines, DNA methylation in promoter regions, which results in gene repression or silencing, was changed in a significant fraction of the genes altered in HD (Ng et al. 2013), although how mutant huntingtin triggers DNA methylation is currently unknown.

Gene expression is also influenced by non-coding RNAs. In HD human brain, miRNA deregulation has been reported (Johnson et al. 2008)(Packer et al. 2008) (Martí et al. 2010). Moreover, huntingtin has been found in RNA structures such as P bodies (Savas et al. 2008), stress granules (Ratovitski et al. 2012) or dendritic RNA granules (Savas et al. 2010), where it could influence protein expression at a post-transcriptional level. In *Drosophila* models of the related polyglutamine disease spinocerebellar ataxia type 3, expression of an untranslated CAG triplet expansion was sufficient to confer toxicity (Li et al. 2008). RNA toxicity mechanisms include aberrant protein-RNA interactions and sequestration of proteins but also the hairpin secondary structure formed by CAG RNAs resemble dsRNA structures that are substrates for Dicer (Handa et al. 2003), cleaving them into shorter repeat that silence specific genes (Krol et al. 2007). Cleaved RNAs from CAG-expanded huntingtin may also become neurotoxic through Ago2-mediated gene silencing of CTG-containing genes (Bañez-Coronel et al. 2012).

### ***Impairment of protein degradation systems: UPS and autophagy***

Two major degradation pathways exist to degrade intracellular proteins: the ubiquitin proteasome system (UPS), that efficiently degrades wild-type huntingtin; and the autophagy-lysosome system which seems to be important in degrading the expanded mutant forms (Ravikumar et al. 2002)(Ravikumar et al. 2004)(Shibata et al. 2006).

Although most efforts have focused on finding strategies to upregulate these systems in order to reduce the levels of mutant protein, the influence that mutant huntingtin has in UPS and autophagy has been also a matter of research.

Early reports described an impairment in proteasome activity as a consequence of the expression of polyglutamine-expanded huntingtin (Bence et al. 2001)(Jana et al. 2001)(Bennett et al. 2005)(Verhoef et al. 2002), a phenomenon that might be explained by either the sequestration of components of the UPS into inclusions (DiFiglia et al. 1997)(Davies et al. 1997)(Waelter et al. 2001), or by the interaction between the proteasome and aggregation-resistant forms of huntingtin (Holmberg et al. 2004)(Venkatraman et al. 2004).

Conversely, some groups did not observe deficits in UPS activity in HD (Bett et al. 2009)(Maynard et al. 2009)(Schipper-Krom et al. 2014a). Studies in single neurons and in mouse models have addressed this contradiction by revealing that an initial UPS impairment is followed by its normalization coinciding with the appearance of inclusions, suggesting an adaptive mechanism (Mitra et al. 2009)(Ortega et al. 2010). More recently, it was shown that proteasomes can completely degrade expanded polyglutamines (Juenemann et al. 2013) which, together with the observation that proteasomes can be dynamically recruited to inclusions without affecting their activity (Schipper-Krom et al. 2014b), are in favour of a competent UPS in HD.

Although an increased number of autophagosomes was described in HD models (Kegel et al. 2000), autophagosome formation is not affected by either mutant or wild type huntingtin (Zheng et al. 2010). A closer look to the autophagic machinery revealed that, although formed, HD autophagosomes cannot optimally sequester substrates (Martinez-Vicente et al. 2010). This might be explained by the recently hypothesised role of wild type huntingtin as a protein scaffold to recruit the autophagy machinery in selective autophagy, although the consequences of the triplet expansion in this scaffold function has not been addressed (Ochaba et al. 2014). Additionally, it has been proposed that HD autophagosomes have impaired axonal transport (Wong and Holzbaur 2014), which leads to inefficient autophagosome-lysosome fusion and decreased degradation of autophagosome content (Ravikumar et al. 2005)(Jahreiss et al. 2008).

### ***Altered synaptic plasticity and neuronal homeostasis in HD***

Neuronal and synaptic abnormalities are early pathological events in HD (Usdin et al. 1999)(Milnerwood et al. 2006)(Cummings et al. 2006). Neuronal homeostasis might be compromised by decreased transcription of essential genes in neurotransmission and signalling but also by defects in the delivery of proteins and organelles along their axons. Pathogenic huntingtin inhibits fast axonal transport of organelles (Li et al. 2001)(Szebenyi et al. 2003)(Lee et al. 2004)(Gunawardena et al. 2003)(Trushina et al. 2004), a phenomenon that has been explained by aggregates blocking axons (Li et al. 2001)(Lee et al. 2004), aggregate sequestration of motor proteins (Gunawardena et al. 2003)(Trushina et al. 2004) or loss of function of wild type huntingtin (Gunawardena et al. 2003)(Trushina et al. 2004). Huntingtin facilitates vesicle trafficking by serving as a scaffold between cargoes, microtubules and motor proteins, such as dyneins or kinesins (Caviston et al. 2007)(Colin et

al. 2008), an interaction mediated through huntingtin associated protein 1 (HAP1) which appears to be disrupted in disease (Gauthier et al. 2004)(McGuire et al. 2006). Polyglutamine-expanded huntingtin may also have an indirect effect by enhancing JNK3 phosphorylation of kinesin heavy chain, which disrupts its binding to microtubules in cellular and animal models of HD thereby perturbing fast axonal transport (Morfini et al. 2009).

Axonal transport is required to correct delivery to neuronal membranes to ensure synaptic transmission. In HD, a failure delivery of receptors such as GABA(A) or AMPA receptors, inhibits synaptic excitability. HAP1 is the scaffold linking these receptors to the kinesin motor KIF5 and this interaction is interrupted by mutant huntingtin (Twelvetrees et al. 2010)(Mandal et al. 2011)(Yuen et al. 2012). Mutant huntingtin also inhibits cortical transport and release of BDNF (Gauthier et al. 2004), or the retrograde transport in the striatum of its receptor TrkB (Liot et al., 2013), necessary to promote survival signals in the cell body.

Medium-sized spiny neurons in the striatum experience the most prominent degeneration in HD. The observation that MSN were selectively affected by glutamatergic signals (Coyle and Schwarcz 1976)(McGeer and McGeer 1976)(Beal et al. 1986)(Beal et al. 1991) led to the hypothesis that striatal neurons in HD could be harmed by excessive neurotransmission, mainly through glutamate stimulation of NMDA receptors, resulting in neuronal cell death via a process termed excitotoxicity.

Alterations in the levels of the different subunits of postsynaptic NMDAR in striatum could explain their aberrant activity in HD (Cepeda et al. 2001)(Ali and Levine 2006)(Fan et al. 2007)(Benn et al. 2007), which may predispose striatal neurons to excitotoxic damage (Laforet et al. 2001)(Zeron et al. 2002). In addition, mutations in HD might also affect trafficking of NMDAR in striatal neurons (Fan et al. 2007)(Marco et al. 2013). But also, the balance between synaptic (pro-survival) and extrasynaptic (detrimental) NMDAR activity is altered in HD (Okamoto et al. 2009)(Milnerwood et al. 2010). Excitotoxicity might result from an increase glutamate release or from impaired uptake and clearance, as downregulation of GLT1 glial glutamate transporter has been observed in HD (Liévens et al. 2001)(Shin et al., 2005)(Estrada-Sánchez et al. 2009). Therapeutic agents targeting excitotoxicity may act directly on NMDAR, such as memantine (Okamoto et al. 2009)(Milnerwood et al. 2010), or modulate levels of excitatory neurotransmitters, such as 3-hydroxikynurenine and quinolinic acid, both metabolites of the kynurenine pathway, the major tryptophan degradative pathway, which is perturbed in HD (Giorgini et al. 2005)(Guidetti et al. 2006)(Zwilling et al. 2011)(Campesan et al. 2011).

### ***Mitochondrial dysfunction in HD***

Altered mitochondrial function resulting in defects in ATP production,  $\text{Ca}^{2+}$  buffering capacity and apoptosis is associated with neurodegeneration in HD (Sawa et al. 1999)(Panov et al. 2002). Some evidence suggests that mutant huntingtin can interact with the outer mitochondrial membrane resulting in mitochondrial calcium abnormalities (Panov et al. 2002)(Choo et al. 2004). Mutant huntingtin also interferes with normal organellar axonal transport and can therefore reduce transport of mitochondria to synapses, as well as ATP production (Orr et al. 2008)(Song et al. 2011)(Shirendeb et al. 2012).

Decreased transcription of mitochondrial genes may also contribute to mitochondrial defects, such as repression of PGC1- $\alpha$ , a nuclear co-activator that regulates the expression of genes that mediate mitochondrial biogenesis and respiration (Cui et al. 2006), or depletion of the enzyme necessary for



synthesizing cysteine which maintains mitochondrial homeostasis (Paul et al. 2014). Also, transport of proteins into mitochondria could be defective, since huntingtin interacts and inhibits TIM23, a component of the inner mitochondrial membrane transport complex and this may contribute to respiratory dysfunction and neuronal cell death (Yano et al. 2014).

Mitochondria are dynamic organelles that undergo fusion-fission cycles in response to stimuli and metabolic demands. Fragmentation leads to caspase activation and apoptosis, and therefore inhibiting mitochondria fission delays cell death (Youle and Karbowski 2005). Expanded huntingtin interfere with mitochondrial dynamics and interacts with a central regulator of protein fission, dynamin-related protein 1 (Drp-1), increasing its enzymatic activity and mitochondrial fragmentation. Conversely, overexpression of a negative form of Drp-1 or fusion-promoting enzymes inhibit mutant huntingtin-induced mitochondrial fragmentation and toxicity (Wang et al. 2009)(Song et al. 2011)(Shirendeb et al. 2012) and selective Drp1 inhibitors have proven beneficial to slow down disease progression in several HD models (Guo et al. 2013).

A consequence of mitochondrial malfunction is the aberrant production of reactive oxygen species (ROS), which, in turn causes more damage to mitochondria. Post-mortem brain of HD patients and experimental models of HD show evidence of oxidative damage (Perluigi et al. 2005)(Stoy et al. 2005)(Sorolla et al. 2008). Therefore, antioxidants are currently being tested to ameliorate levels of ROS and to help in mitochondrial dysfunction.

#### ***Cell-to-cell transmission of aggregates***

Emerging evidences suggest that prion-like transmission from cell to cell of proteins like tau or alpha-synuclein spreads these disease-associated proteins to different brain regions (Lee et al. 2010)(Guo and Lee 2014). This “infectious” property has been also associated with polyglutamine proteins, where internalization of exogenous polyglutamine aggregates serves as seeds for nucleating aggregation of cytoplasmic soluble polyglutamines (Ren et al. 2009). This first report suggested that aggregates are internalized from the extracellular space but more recently, cell-to-cell transfer of aggregates through tunnelling nanotubes, actin-rich membrane bridges that connect cells and mediate the transfer of cytoplasm content (Rustom et al. 2004), such as prions (Gousset et al. 2009), were suggested as an alternative route (Costanzo et al. 2013).

In vivo studies have shed some light into this hypothesis. hESC-derived neurons that were integrated into corticostriatal organotypic brain slices of a R6/2 mouse or injected into the cortex, acquired mutant huntingtin aggregates after 2 and 4 weeks respectively, which correlated with alterations in neuron integrity. Further, corticostriatal co-cultures revealed that mutant huntingtin spread from R6/2 cortex to wild type MSNs in the striatum, but not in the opposite direction (Pecho-Vrieseling et al. 2014), suggesting that propagation occurred in a pre- to post-synaptic path, which was confirmed by using inhibitors of synaptic vesicle fusion (Pecho-Vrieseling et al. 2014).

#### ***Astrocyte and microglial dysfunction in HD***

Although huntingtin aggregates are more prominent in neurons than in non-neuronal glial cells (Shin et al., 2005), probably due to the lack of cell division in neurons or to a less efficient protein homeostasis system (Tydlacka et al. 2008), glial cells also contribute to disease in HD and reactive gliosis is observed in many HD mouse models (Reddy et al. 1998)(Lin et al. 2001)(Yu et al. 2003) and postmortem brains of HD patients (Myers et al. 1991)(Sapp et al. 2001).

Astrocytes are the major type of glia. They provide support to neurons and enable uptake of extracellular glutamate preventing excitotoxicity. In an effort to discern the role of astrocytes in HD pathology, an N-terminal huntingtin with 160Q was selectively expressed in astrocytes. Despite no obvious degeneration of glia or neurons, these mice developed late-onset neurological symptoms, which correlated with reduced levels of the GLT-1 glutamate transporter (Bradford et al. 2009). When mutant huntingtin was expressed in both astrocytes and neurons, it worsened the phenotype relative to neuronal-only expression, confirming the contribution of astroglia to disease (Bradford et al. 2010).

Additional defects might contribute to pathology, such as impaired secretion from HD astrocytes of the chemokine CCL5 (Chou et al. 2008) or BDNF (Wang et al. 2012). Also, striatal astrocytes from R6/2 and Q175 HD mouse models showed reduced levels of Kir4.1 K<sup>+</sup> channels, which led to increased extracellular K<sup>+</sup> and neuronal excitability, while viral delivery of Kir4.1 attenuated R6/2 mice phenotype (Tong et al. 2014).

Growing evidence implicates neuroinflammation in neurodegeneration. In HD, increased secretion of pro-inflammatory cytokines and chemokines has been reported in late but also in early presymptomatic gene carriers (Tai et al. 2007)(Björkqvist et al. 2008)(Wild et al. 2011), suggesting that this is not only a reactive process but an active player in disease progression.

Huntingtin is expressed in immune cells resulting in cell-autonomous microglial activation and secretion of pro-inflammatory cytokines, as a consequence of elevated transcription of myeloid lineage-determining factors PU.1 and C/EBPs (Crotti et al. 2014). In the peripheral immune system, mutant huntingtin also impacts on inflammatory responses through inhibition of NF-kappa B signalling (Trager 2014). Signalling through CB2 cannabinoid receptors might also explain inflammation in HD (Palazuelos et al. 2009)(Bouchard et al. 2012). In addition, both microglia and peripheral cells expressing mutant huntingtin showed reduced migration in response to chemotactic signals (Kwan et al. 2012b). Moreover, bone marrow transplantation with wild type cells restored the levels of cytokines and chemokines, and partially suppressed pathology in HD mouse models (Kwan et al. 2012a).

### **Interplay between mutant huntingtin and other aggregate prone proteins**

Mutant huntingtin is also associated with other brain pathologies.  $\alpha$ -synuclein, the component of Lewy bodies in Parkinson's disease, is found close to huntingtin aggregates (Charles et al. 2000) and excess  $\alpha$ -synuclein expression is associated with increased mutant huntingtin aggregation (Furlong et al. 2000)(Herrera and Outeiro 2012). In agreement with these findings, when  $\alpha$ -synuclein was knocked out in a R6/1 mouse, the number of inclusions was reduced and the disease progression attenuated (Tomás-Zapico et al. 2012). Overexpression of  $\alpha$ -synuclein has a negative effect on autophagy (Winslow et al. 2010) and worsens the disease phenotype in R6/1 and N171-82Q mouse models (Corrochano et al. 2012). Conversely, its depletion was beneficial and correlated with an increase in autophagy in these mice which explains the crosstalk between these two diseases (Corrochano et al. 2012). Since mutant huntingtin is an autophagy substrate, these observations are a likely major contributor to the cross-talk between  $\alpha$ -synuclein and huntingtin aggregation, as opposed to any obvious cross-seeding.

An imbalance in the levels of tau isoforms containing either three or four microtubule binding repeats (3R or 4R) with an increased in the 4R/3R ratio is sufficient to cause neurodegeneration. This

relation is increased in HD mice as a consequence of splicing defects and enhances HD pathology (Fernández-Nogales et al. 2014). Tau phosphorylation is also affected by HD mouse models, which might have consequences in HD progression (Blum et al. 2014).

#### **4. Therapy for Huntington's Disease**

##### ***HD as a tractable therapeutic problem***

Though Huntington's disease is rare, it does receive a great deal of research attention. One reason for this is that it has some features which make it more likely to be a tractable problem than other neurodegenerative conditions. Firstly, the autosomal dominant nature of the condition means that the diagnosis is almost definitive and can be made prior to death. This means that it is possible to accurately model and study the disease *in vitro* and *in vivo*. Perhaps more importantly, one can be sure the patients are suffering from a reasonably homogeneous condition. This is not the case in other dementing illnesses where the diagnosis is seldom definitive and post mortem analysis often shows a mix of pathologies. Secondly, the familial nature of the condition means that diagnosis can be made prior to symptom onset. This is a potentially crucial advantage as it means therapy can begin prior to major neuronal loss, by which point in the illness it may be more difficult to slow progression and impossible to correct existing deficits. Lastly, the Huntington's community of patients, their families and their doctors have a history of co-operation which has made large scale clinical trials possible. This is clear not only from trials that have taken place but also long term longitudinal studies of disease progression which have provided rich data sources to inform the design of future trials, particularly with regards to appropriate trial endpoints (Tabrizi et al., 2013).

##### ***Current treatment for HD***

There are no known disease-modifying drugs currently available for HD. Treatment is symptomatic only. The only drug with a licensed indication for HD in the UK is tetrabenazine, where it is used to treat choreaform movements. Trials of cholinesterase inhibitors used to treat the cognitive problems seen in Alzheimer's disease have been largely negative in HD (Cubo et al., 2006). Psychiatric symptoms, which are often the most troubling for patients, are often treated with standard drug treatments used in non-HD patients. For example, psychosis is treated with atypical antipsychotics and depression with SSRI or SNRI antidepressants (Phillips et al., 2008). With the exception of one open label trial with venlafaxine, these treatments are supported largely by case studies or small series (Holl et al., 2010). The current care of people with HD involves many paramedical disciplines, including speech and language therapy, physiotherapy, nursing and social care.

##### ***Therapeutic trials based on potential pathogenic mechanisms***

###### **i) Gene silencing**

Silencing the expression of the mutant huntingtin gene is attractive, as one might expect it to provide an effective treatment by dealing with the pathology at source. Indeed, trials in rodents have found the approach to be efficacious in ameliorating symptoms and pathology either using RNA interference (Drouet et al., 2009)(Stanek et al. 2014) or antisense oligonucleotides (Kordasiewicz et al. 2012). Though attractive, this approach

has a number of potential difficulties. These include allele specificity, off target effects and delivery. Recent non-human primate trials have shown promising safety data, and a small safety trial of antisense oligonucleotides in HD patients is planned for 2015 (Grondin et al. 2012)(McBride et al. 2011).

ii) Anti-apoptotics / caspase inhibition

The tetracycline antibiotic minocycline is a caspase inhibitor, though like many of the compounds described here has pleiotropic mechanisms of action, including antioxidant and cytokine modulating properties. It was initially shown to prolong life expectancy in a mouse model of Huntington's disease, though subsequent work in the same mouse model was not encouraging (Chen et al., 2000, Menalled et al., 2010). Nevertheless, following small safety trials, a larger trial of patients with an endpoint of change in total functional capacity compared to historical controls suggested no benefit of minocycline. This highlights the importance of careful reproduction of preclinical data before moving in to clinical trials.

iii) Transglutaminase inhibition

The glutamine residues in huntingtin are cross linked by transglutaminase. Transglutaminase inhibitors such as cystamine have produced promising results in mouse models of the disease (Dedeoglu et al., 2002)(Karpuj et al. 2002). A safety and dose finding study of a cystamine dimer, cysteamine, has been carried out (Dubinsky and Gray, 2006) and a larger trial involving 96 HD patients is underway.

iv) Mitochondria, oxidative stress and excitotoxicity

Using antioxidants to decrease oxidative stress has been a putative therapeutic strategy for a number of neurodegenerative diseases. This is another example where treatment of mouse models suggested benefit, in this case with the NMDA receptor antagonist remacemide and the antioxidant coenzyme Q10 (Ferrante et al., 2002). Unfortunately these results were not recapitulated in large clinical trials of patients (2001). More recently, in the largest proposed trial in HD to date, the 2CARE study of high dose coenzyme Q10, was stopped early due to a combination of futility and safety concerns. Other clinical trials with other NMDA receptor antagonists have also been disappointing (Kremer et al., 1999) as have trials of creatine, a potential antioxidant with previous positive results in mice. Trials of creatine in symptomatic patients have been disappointing, but further trials have been undertaken in at-risk individuals with some more positive findings on imaging (Rosas et al., 2014).

v) Up-regulating autophagy

Autophagy up-regulation using a variety of drugs have shown amelioration of the HD phenotype and pathology in cellular, fly, fish and mice models, reviewed in (Hochfeld et al., 2013). Conversely, inhibition of autophagy has been shown to worsen phenotypes, including using antioxidants which are autophagy inhibitors, and this may provide an explanation for the relative failure of antioxidant based strategies (Underwood et al., 2010). The repertoire of drugs which up-regulate autophagy has been expanded and includes compounds such as rilmenidine which have a benign side effect profile and long

records of safe human use. A safety trial of rilmenidine is currently underway in HD patients in Cambridge, UK.

#### vi) Transplantation

Once symptoms are manifest, it may be difficult to reverse deficits with drug treatment as neurons have been lost. One approach to correcting this is to transplant new neuronal tissue. This approach is another where some promise has been shown in animal models of the disease (Dunnett et al., 1998). Early human studies have shown potential for graft survival, though a recent long term follow up was less encouraging and mutant huntingtin is found in transplanted tissue (Barker et al., 2013, Cicchetti et al., 2014). The variable results in terms of graft survival, safety and outcome may reflect differences in protocol and procedure which may lead to more successful trials now proof of principle has been established.

#### vii) Other clinical trials

Other large trials have been reported in HD. Latrepirdine (Dimebon) was originally synthesised as an antihistamine and showed some early promise as a treatment for Alzheimer's disease. Results from the relatively large DIAMOND trial in HD patients suggested some improvement in cognition, but the subsequent larger HORIZON trial showed no benefit and the company involved is no longer taking this compound forwards as a potential therapy (Kieburtz et al., 2010). Other compounds which may help with symptoms but which may not be disease modifying have also been studied in large scale trials. Pridopidine is a drug which may have a beneficial effect on movement via its effect on dopamine signalling. A large recent trial did not reach statistical significance for the primary motor endpoint, but did suggest some promise in motor scores overall. Non-drug strategies are also being pursued, for example recent trials of physical activity and rehabilitation in HD patients have been reported (Busse et al., 2013).

The increase in understanding of the basic science of HD has yet to translate into an effective disease modifying therapy. Feasibility of large scale trials of various sorts has been demonstrated and a wide variety of approaches are currently being pursued, which one hopes will start to bring benefits in the near future.

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**Figure Legend:**

Figure 1: Schematic of selected mechanisms of pathogenesis in Huntington's disease

### 3. Altered protein homeostasis:

- Proteasome dysfunction has been proposed as a mechanism of pathogenesis in HD
- Autophagic degradation is affected due to inefficient cargo sequestration or autophagosome transport

### 2. Transcriptional dysregulation:

- Altered transcription of essential genes such as BDNF
- Chromatin modifications

### 1. Aggregate formation:

- Mutant huntingtin with a polyglutamine expansion is cleaved in toxic fragments
- Toxicity may arise from the oligomeric soluble species or from the insoluble inclusions that sequester essential proteins

### 7. Neuroglia dysfunction:

- Microglia secretes pro-inflammatory cytokines and chemokines
- Impaired glutamate uptake by astrocytes

### 6. Axonal transport defect:

- Impaired organelle transport: mitochondria, autophagosomes, synaptic vesicles etc
- Impaired synaptic transmission (defective delivery of neurotransmitter receptors)

### 4. Mitochondrial dysfunction:

- Defective Ca homeostasis
- Altered mitochondrial protein import
- Impaired mitochondrial dynamics (increased fragmentation)
- Aberrant ROS production
- Decreased ATP production

### 5. Altered synaptic plasticity

- Excitotoxicity due to altered postsynaptic NMDAR, glutamate uptake etc
- Deficient delivery of synaptic vesicles and postsynaptic receptors

