

# Molecular Pathogenesis of Huntington's Disease

Huntington's disease (HD) is a genetic neurodegenerative disease with a complex set of symptoms and an insidious progression that continues until death. The cause of HD is the pathological expansion of an unstable (CAG<sub>n</sub>) trinucleotide repeat within the coding region of the HD gene (for references, see 1). The CAG repeat codes for a polyglutamine repeat in the huntingtin (htt) protein. To date, 9 other 'polyglutamine repeat' diseases have been identified, including spinobulbar muscular atrophy (SBMA), several of the spinocerebellar ataxias (SCA1,2,3,6,7 and 17) and dentatorubralpallidolysian atrophy (DRPLA). In each of these diseases, the protein carrying the mutation and also the distribution of neuronal loss is different. (The different protein context in each disease is likely to be responsible for the difference in the patterns of neurodegeneration). However, the fact that all of these diseases are caused by a similar mutation, coupled with the fact that they are all are dominant, (except SBMA that is X-linked), adult-onset, progressive neurodegenerative diseases, suggests that they may have a common underlying pathological mechanism.

## Htt, a protein with elusive function

The precise function of htt remains unknown. It is a large cytoplasmic protein found loosely associated with synaptic vesicles in nerve terminals, and with microtubules in dendrites. It has no homology with any other protein and no distinguishing features that predict its biological function: There are no membrane spanning domains, it does not appear to have enzymatic activity and is not a structural protein. Htt is essential for mammalian development, since deletion of both copies of the HD gene retards the development of the embryo and kills it in mid-gestation. However, when only one copy of HD is knocked out, growth and development appears to be normal. Further, although deleting one copy of the gene causes subtle

deficits in adult mice, these are very mild compared with those seen in mice carrying the HD mutation<sup>(2)</sup>. Since patients who are homozygous for the HD gene are neurologically normal until the onset of their disease, it seems that the expanded polyglutamine repeat does not interfere with the normal function of htt. Rather, HD is caused by a novel *gain* of function of the mutant protein.

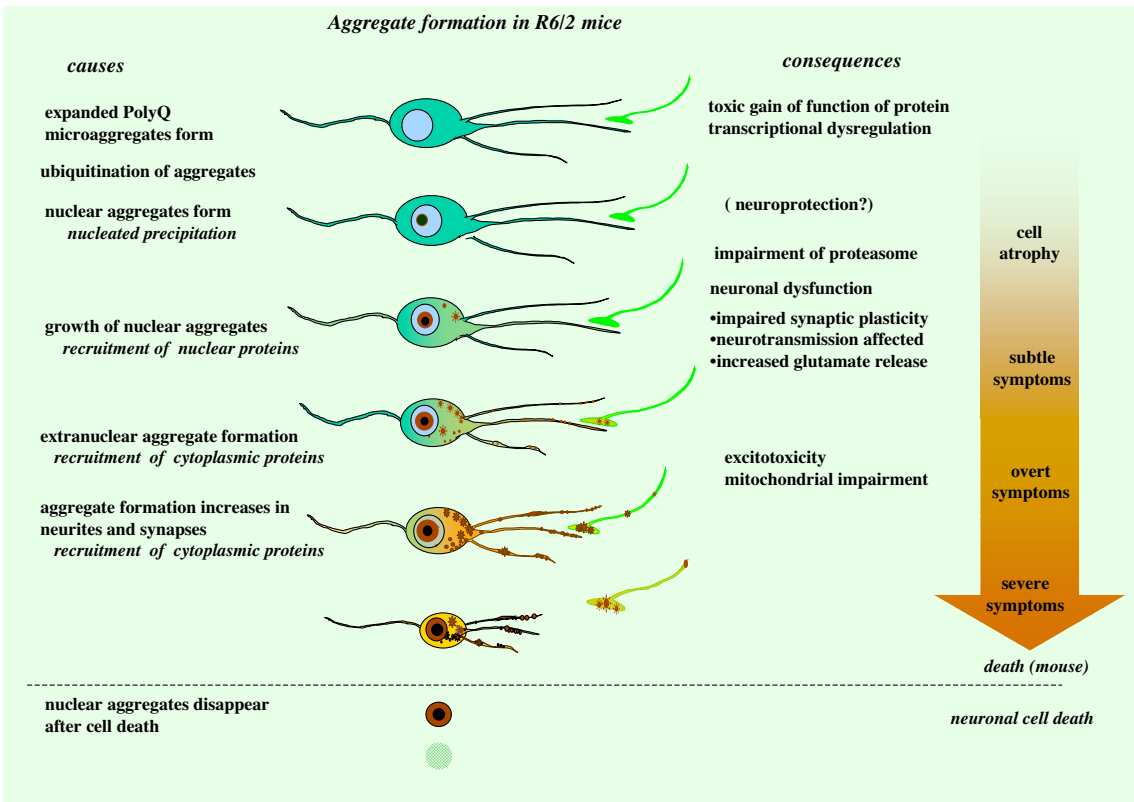
## Abnormal protein-protein interactions with mutant htt

A number of proteins are known to interact with htt<sup>(3)</sup> (Table 1). Some of these interactions change when the protein carries a polyglutamine repeat in the pathological range. For instance, interactions of htt with htt-associated protein 1 (HAP1) are increased, and interactions with htt-interacting protein 1 (HIP1) are decreased with increased polyglutamine length<sup>(4)</sup>. This suggests that a change in the interaction between htt and the interacting protein(s) may also contribute to the pathology underlying HD. For example, HIP1, when over-expressed in neurons, is neurotoxic<sup>(4)</sup>. If mutant htt has a reduced binding capacity for HIP1, this may result in an endogenous toxicity mediated by increased intracellular HIP1. The precise roles of some of the proteins with which htt interacts are themselves unknown. However, their putative roles give strength to the possibility that changes in their interactions with htt may contribute to the pathogenesis in HD (Table 1).

## Why do striatal neurons degenerate in HD?

The medium-sized spiny GABAergic neurons of the striatum (caudate nucleus and putamen) degenerate in HD, and atrophy of the caudate and putamen is the pathological hallmark of HD. Striatal degeneration is the first and most obvious neuropathology in the early-grade HD brain. However, later, profound loss of neurons in other regions (particularly the neocortex) is also seen.

Dr Jenny Morton is a Senior University Lecturer. She has been working on HD since 1990, first with neurochemical models, and more recently with transgenic mouse models of HD. Her recent work has been aimed at characterising the neurological phenotype of HD mouse models, paying particular attention to the progression of cognitive deficits and to identifying potential biomarkers for HD.



Interestingly, the pattern of degeneration in HD does not directly reflect the expression pattern of htt. Although htt is relatively abundant in striatal neurons, it is widely expressed throughout the brain, for example in cortex (pyramidal neurons), cerebellum (Purkinje cells) and thalamus. While a number of proteins are preferentially distributed in the striatum; however, as yet there is no evidence to suggest that any of them is directly responsible for the selective degeneration of striatal neurons in the early stages of HD.

### Increased striatal vulnerability in HD

The striatum is the main target for glutamatergic output neurons from both the cortex and the thalamus. Striatal neurons are sensitive to glutamate, and excitotoxic neurodegeneration can be induced by injecting the glutamate agonist quinolinic acid, directly into the striatum. However, excitotoxicity in the striatum can also be induced indirectly, by using metabolic poisons such as 3-nitropropionic acid (3-NP)<sup>(5)</sup>. 3-NP depletes energy levels in neurons and makes them more vulnerable to excitotoxicity mediated by endogenous levels of glutamate. The striatal neurons are also the main target for dopaminergic input from the substantia nigra. Dopamine modulates the toxicity of endogenous glutamate, since removing the dopamine input to the striatum markedly reduces the size of 3-NP lesions<sup>(6)</sup>. There is compelling evidence from both animals and human studies to suggest that energy levels are compromised in HD<sup>(1)</sup>. There is also evidence from animal studies that glutamatergic activity of the corticostriatal pathway is abnormal in HD mice<sup>(7)</sup>. Thus, either a change in energy levels and/or an change in the activity of glutamate input could increase the vulnerability of striatal neurons in HD.

### Abnormal protein aggregation in HD

A number of transgenic mouse models have been made for HD. These have been particularly valuable for studies

of pathology and behavior (for references, see 1). Ubiquitinated protein aggregates containing htt fragments and other key proteins were first observed in the R6/2 mouse model of HD<sup>(1)</sup>. However, they have now been seen in post mortem brains from HD patients, as well as in patients and mouse models of other polyglutamine repeat diseases. These aggregates, also known as inclusions, are found in the nuclei as well as the neuropil of affected neurons. There is much debate about the role of these aggregates - in particular whether or not they are neurotoxic or protective - there is no doubt that they are a hallmark of HD. In fact, when all of the evidence is reviewed, it seems highly likely that the aggregates play a role in polyglutamine toxicity<sup>(8)</sup>.

In the R6/2 line of HD mice, aggregates appear first in the nuclei, and then in axons, dendrites and synapses of neurons (Fig.1). Their appearance correlates with the onset of symptoms. However, the progressive and changing nature of the neurological phenotype in the R6/2 mouse suggests that the aggregates may also have changing roles<sup>(1,9)</sup>. Aggregation of abnormal and potentially toxic htt protein may initially be beneficial or benign, simply because the toxic protein is removed from the cell milieu. However, it seems likely that if mature inclusions recruit essential cell proteins (e.g. heat shock proteins, proteasome components, /-synuclein), then the presence of the aggregate itself would be deleterious to cellular function. Further, the presence of aggregates of protein in axons, dendrites or synapses could directly impair essential functions of these structures.

In symptomatic R6/2 mice, ubiquitinated aggregates are found in virtually all neurons. However, in post mortem HD brain, nuclear aggregates are relatively sparse in striatal neurons, but are predominant in the cortical neurons (for references, see 1). The cause of this unexpected distribution of aggregates is not known. It may be that when striatal neurons degenerate, the aggregates disappear. Thus, the lack of striatal aggregates in post

**Table 1** Some examples of proteins that interact with huntingtin (for others, see 1, 3)

Interacting protein		PolyQ-length dependent interaction?	Functional role
AKT/PKB	serine theronine kinase	no	cell signaling
CA150	co-activator 150	no	transcriptional activator
CBP	cAMP response element binding protein	yes (increased binding)	transcriptional co-activator
CIP4	Cdc42-interacting protein 4	yes (increased binding)	signal transduction
CtBP	co-repressor C-terminal binding protein	yes (decreased binding)	transcription factor
Grb2	growth factor receptor-binding protein 2	not known	growth factor receptor-binding protein
HAP1	huntingtin-associated protein 1	yes (increased binding)	membrane trafficking
HAP40	huntingtin-associated protein 40	not known	??
HIP1	huntingtin-interacting protein 1	yes (decreased binding)	endocytosis (proapoptotic)
HIP14/HYP-H	huntingtin-interacting protein 14	yes (decreased binding)	receptor trafficking, endocytosis
HYP J ( /-adaptn C)		yes (decreased binding)	endocytosis
HYPA/FBP11		not known	pre-mRNA splicing factor
N-CoR	nuclear receptor co-repressor	yes (increased binding)	transcriptional regulator
NFkB		not known	transcriptional factor
P53		no	transcriptional factor
PACSIN1	protein kinase C and casein kinase substrate in neurons 1	yes (increased binding)	endocytosis
PSD-95	post-synaptic density protein 95kDa	yes (decreased binding)	synaptic scaffolding protein
RasGAP		Not known	Ras GTPase-activating protein
SH3GL3		yes (increased binding)	endocytosis
Sin3a		yes (increased binding)	transcriptional repressor
Sp1		yes (increased binding)	transcriptional factor
TAFII-130	TBP-associated factor II 30	no	transcriptional factor

mortem striatum may merely reflect striatal neuronal cell loss. Alternatively it could mean that in HD, cortical dysfunction occurs first, and drives striatal neurodegeneration by the excitotoxic mechanisms outlined above.

### Proteolysis and proteasome dysfunction

In normal cells, abnormally folded proteins are conjugated with multiple ubiquitin molecules and targeted for degradation by the proteasome, a multicatalytic protease complex. The protein aggregates seen in HD are ubiquitinated, suggesting that the proteins have been marked for degradation by the proteasome. The fact that the proteins are not degraded, but aggregate instead, implies an impairment of the cell chaperone and proteasome machinery. Htt is cleaved by a number of different proteases, including some caspases (proteases that are key mediators of programmed cell death pathways) and calcium-dependent proteases such as calpain<sup>(10)</sup>. The N-terminal fragments released by such cleavage appear to be more toxic than the full-length protein<sup>(11)</sup>. Further, although they do not appear to impair proteasome function greatly in cellular models, these fragments impair the cells' normal response to stress and toxicity<sup>(12)</sup>. However, proteasome components have been found within aggregates in HD cell models, suggesting a direct involvement in the aggregate pathology<sup>(13)</sup>. Proteasomal dysfunction would have both direct and indirect effects on a cell, firstly because the mutant htt is not being degraded and secondly because the proteasome would also not be able to degrade other misfolded proteins that might have deleterious effects on cell function. Proteasome dysfunction would have a further 'knock-on' effect in the cell, because the proteasome plays a central role in the turnover of normally-folded but short-lived proteins in the cell. Thus proteasome dysfunction could mediate multiple pathways of dysregulation of normal cell function.

### Transcriptional dysregulation in HD

A large number of genes are abnormally expressed in HD mouse models (for references, see 1). The protein products of these genes are found in key molecular systems, including neurotransmitter pathways (particularly dopamine), intracellular signaling pathways and calcium homeostasis. However, at present it is not possible to tell which of the many genes that are dysregulated in HD are involved in the primary pathology and which are secondary. While some of the dysregulated genes have been shown to result in altered protein expression (e.g. those coding for DARPP-32 and complexin II (CPLXII)), most have not been studied in depth.

### Abnormalities in synaptic transmission

As well as changes in neurotransmitters and receptors, modulators of transmission have also been implicated in HD. For example, CPLXII is decreased in both HD post mortem brain and HD mouse brains<sup>(14)</sup>. CPLXII is a modulator of neurotransmitter release, and CPLXII knockout mice exhibit progressive motor and cognitive deficits<sup>(15)</sup>. This suggests that the decrease in CPLXII seen in HD may underlie some of the cognitive and motor deficits. Other evidence suggesting that synaptic dysfunction is one of the earliest changes in HD includes the observation that, as with human patients, symptoms in all of the mouse models appear before there is frank neurodegeneration (for references, see 1). In mice, abnormalities in synaptic plasticity and transmitter release are measurable before overt symptoms appear. Thus it appears that neuronal

dysfunction rather than neurodegeneration might give rise to the earliest symptoms in HD.

### Summary

Although the mutation causing HD appears to be relatively simple, the downstream consequences of the mutation are extremely complex. First, it seems likely that neuronal dysfunction precedes neurodegeneration in both animals and humans. Second, the HD mutation appears to perturb multiple intracellular pathways. Only some of these will be primary responses to the toxic gain of function of the HD mutation. Knowing which of the changes is 'cause' and which is 'effect' will be fundamental to advancing our understanding of HD to a level where treatment becomes a realistic possibility.

### References

- Bates GP, Harper PS and Jones AL. (2002) Huntington's disease, 3rd edition. Oxford University Press, Oxford
- Catteneo E, Rigamonti D, Goffredo D, Zuccato C, Squitieri F and Sipione S. (2001) Loss of normal huntingtin function: new developments in Huntington's disease research. *Trends in Neurosci* 24:182-8
- Harjes, P. and Wanker, E. (2003) The hunt for huntingtin function: interaction partners tell many different stories. *Trends in Biochem. Sci.* 28: 425-4433
- Hackam AS, Yassa AS, Singaraja R, Metzler M, Gutekunst CA, Gan L, Warby S, Wellington CL, Vaillancourt J, Chen N, Gervais FG, Raymond L, Nicholson DW, Hayden MR. (2000) *Huntingtin interacting protein 1 induces apoptosis via a novel caspase-dependent death effector domain.* *J Biol Chem.* 275: 41299-308.
- Albin RL. (2000) *Basal ganglia neurotoxins.* *Neurol Clin.* 18: 665-80.
- Reynolds DS, Carter RJ, Morton AJ. (1998) *Dopamine modulates the susceptibility of striatal neurons to 3-nitropropionic acid in the rat model of Huntington's disease.* *J Neurosci.* 18: 10116-27
- Klapstein GJ, Fisher RS, Zanjani H, Cepeda C, Jokel ES, Chesselet MF and Levine MS. (2001) *Electrophysiological and morphological changes in striatal spiny neurons in R6/2 Huntington's disease transgenic mice.* *J Neurophysiol* 86:2667-77
- Michaelik, A. and Van Broeckhoven, C. (2003) *Pathogenesis of polyglutamine disorders: aggregation revisited.* *Hum. Mol. Genet.* 12: R173-186
- Morton AJ, Lagan MA, Skepper JN, Dunnett SB. (2000) *Progressive formation of inclusions in the striatum and hippocampus of mice transgenic for the human Huntington's disease mutation.* *J Neurocytol.* 29: 679-702.
- Tarlac V, Storey E. (2003) *Role of proteolysis in polyglutamine disorders.* *J Neurosci Res.* 74: 406-16
- Zhou H, Cao F, Wang Z, Yu ZX, Nguyen HP, Evans J, Li SH, Li XJ. (2003) *Huntingtin forms toxic NH2-terminal fragment complexes that are promoted by the age-dependent decrease in proteasome activity.* *J Cell Biol.* 163: 109-18
- Ding Q, Lewis JJ, Strum KM, Dimayuga E, Bruce-Keller AJ, Dunn JC, Keller JN. (2002) *Polyglutamine expansion, protein aggregation, proteasome activity, and neural survival.* *J Biol Chem.* 277(16): 13935-42
- Waelter S, Boeddrich A, Lurz R, Scherzinger E, Lueder G, Lehrach H, Wanker EE. *Accumulation of mutant huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation.* *Mol Biol Cell.* 2001 May; 12(5): 1393-407.
- Morton AJ, Faull RL, Edwardson JM. *Abnormalities in the synaptic vesicle fusion machinery in Huntington's disease.* *Brain Res. Bull.* 2001 Sep 15; 56(2): 111-7.
- Glynn D, Bortnick RA, Morton AJ. (2003) *Complexin II is essential for normal neurological function in mice.* *Hum Mol Genet.* Oct 1; 12(19): 2431-48.