Huntington's Disease: Pathogenesis and Treatment

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INTRODUCTION

Huntington's disease (HD) is an autosomal, progressive and dominantly-inherited neurodegenerative disorder, characterised by abnormalities of movement, emotion and cognition. It claims its name from the physician, George Huntington, who first described the illness in 1872. The most important pathological feature is selective neuronal loss, primarily in the striatum and cerebral cortex. A major breakthrough occurred in 1993, with the discovery that the mutation causing the disease is the expansion of the CAG trinucleotide repeats in exon 1 of the gene

encoding the protein huntingtin.[1] CAG codes for the amino acid, glutamine, thus the mutation produces an expanded stretch of glutamine residues that are attached to the amino terminal of the huntingtin protein.

HD affects 4-10 per 100,000 individuals of Caucasian origin.[2] A HD genetic test is now available that can show if someone has inherited the mutation. There is usually a delayed onset of the disease with symptoms typically beginning between the ages of 35 and 50; however, the disease

may manifest itself from childhood to old age.[3] Disease onset is insidious. Early complaints include clumsiness, difficulty in balance, minor involuntary movements, lack of concentration and often a depressed or irritable mood. As the disease progresses, chorea (meaning "mad dance") becomes a prominent involuntary movement, along with difficulty in voluntary motor activities, weight loss and difficulties in speech. Cognitive deficits and psychiatric symptoms may prevent patients from fulfilling everyday responsibilities, e.g. retaining employment. Patients in the later stages of the disease generally have severe chorea, resembling monoclonic jerks and progressive disturbances in voluntary movement. Patients at this point are severely rigid, bed-ridden and largely non-verbal with global dementia. They often have difficulty swallowing, which can lead to death, either directly by suffocation or indirectly by starvation.

Death usually occurs 15-20 years after the onset of symptoms. Earlier death may occur as a direct result of the disease. The suicide rate in HD patients is 12.7%, which as indicated by epidemiological and phenomenological evidence, is a result of emotional depression and affective mood disorder caused by the disease rather than a direct reaction to changes in life circumstances. Premature death may also occur from a fall as a result of motor abnormalities. A small number of patients have been known to survive 3-4 decades after onset of the disease.

PATHOGENESIS

Genetics:

The gene for huntingtin is comprised of 67 exons in humans. It is located between the markers D4S127 and D4S180 on chromosome 4p16.3. The gene spans a genomic region of over 200kb.

Huntingtin is a 350kDa protein with no strong homology to known proteins.[3] In normal huntingtin, the number of CAG repeats ranges from 6 to 35, whereas with individuals with the dominant HD mutation, the repeat length varies from 36 to 121.

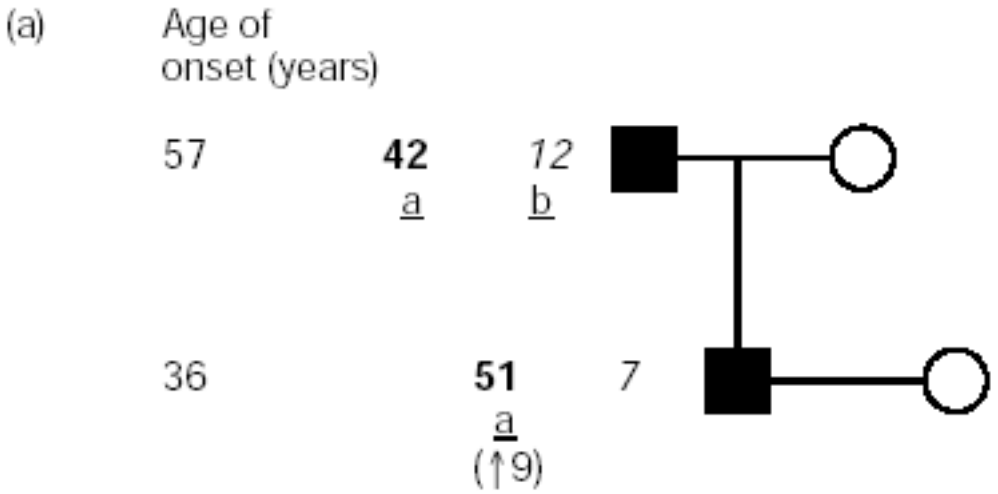
The age of onset of HD is inversely correlated with CAG repeat length. Adult onset generally occurs with repeat lengths of 36-50, while juvenile onset is often seen with greater than 60

repeats. In the past, it was thought that HD had full penetrance, that is, for individuals with the mutant allele the likelihood of them attaining the disease is 100%. However, it subsequently came to light that for individuals carrying an allele with 36-40 repeats there may be less than 100% penetrance and for repeats of 36-37 triplets, it may be in the order of 50%.

A feature that distinguishes the trinucleotide expansion is the non-Mendelian form of inheritance;

the repeat length can grow in each successive generation.[4] This phenomenon known as anticipation occurs with the expansions of the CAG repeat during paternal transmission. In clinical samples of affected parent-child pairs, there was no significant change in the age of onset with maternal transmission, but a mean advance of 8 years occurred with paternal transmission. The net result is a skew towards earlier ages of onset in successive generations of a family driven by paternal transmission.

Figure 1.



(a) Expansion in huntingtin decreases the age of onset in those affected. A representative two-generation pedigree of a HD family. Squares are males; circles are females. Black boxes indicate affected individuals. Numbers in bold represent the CAG repeat number in each allele of the affected family members. Small underlined letters represent the alleles present in father-to-son transmission. The italic green repeat number is a normal allele inherited from the mother. The number in brackets represents the size of the CAG expansion during inheritance.
(b) The relationship between HD pathology and CAG repeat number. In the schematic representation of the HD gene (left), the open bar represents the coding region, huntingtin. The small black bar indicates the position of the CAG repeat stretch located within the N-terminal portion of the coding sequence. The inverted triangle represents an increasing number of CAG repeats. The white base represents unaffected individuals with 6-29 CAG repeats with the black

area representing affected individuals with 36-120 repeats.[4]

Neuropathology:

HD post-mortem neuropathology shows a general atrophy of the brain, but the most prominent atrophy is found in the caudate nucleus and putamen (which together comprise the corpus striatum portion of the basal ganglia). In advanced cases, the total brain weight is reduced by 25-30%. Severe loss of medium spiny neurons, especially those synthesising enkephelin and

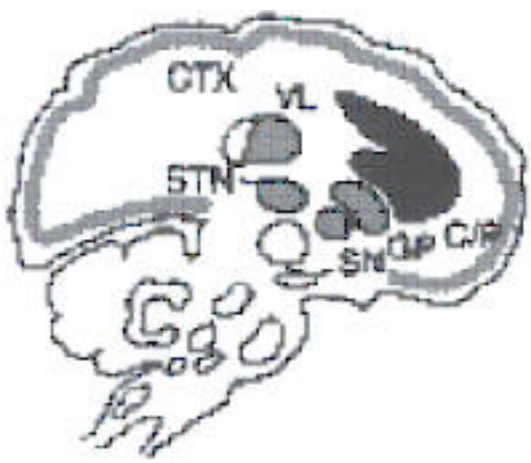
GABA, is most prevalent in the striatum.[3] Substantial neuronal loss is evident in the globus pallidus and subthalamic nucleus. Degeneration in the cerebral cortex is also found with

widespread loss of neurons, especially in layer VI, but also significantly in layers III and V. [5] Thus, the major degeneration, which normally occurs first in the striatum, leads to chorea, and the subsequent loss in the cerebral cortex causes dementia. Inclusion bodies comprised of huntingtin protein, found in the nucleus and cytoplasm in the neurons of HD patients and transgenic mice, indicate that these aggregates may play a major role in the pathogenesis of HD.

There is evidence of regenerative changes and plasticity in the diseased brain as it tries to

compensate for lost neurons.[3] Compared to control brains, surviving neurons in HD patients' brains have more dendrites, a greater density and larger size of dendritic spines, and a greater somatic area.

Figure 2. Affected Brain Regions



Dark regions indicate the major areas of neuronal loss in HD patients. (C/P = caudate/putamen, CTX = cortex, GP = globus pallidus, STN = subthalamic nucleus, VL = ventrolateral thalamic

nucleus, SN = substantia nigra.) [4]

Gain-of-function mutant huntingtin and loss of normal huntingtin function - a hypothesis of the pathogenesis of HD:

Wild-type or normal huntingtin protein is important for development. Mice with targeted deletions in the HD gene demonstrate developmental abnormalities rather than a progressive neurological

disorder. Embryos of huntingtin homozygous knock-out mice die by day 7.5.[6] Studies have shown that a single copy of the huntingtin gene is sufficient for correct brain development, regardless of the length of the CAG repeat. Thus, mutant huntingtin can substitute for its wild-type (normal) counterpart during development, consistent with the fact that homozygous HD patients do not present with developmental defects.

Mutant huntingtin, by virtue of the expanded polyglutamine moiety, has structural similarities to known transcription factors. One of the most specific antibodies recognising the expanded form of huntingtin is the IC2 antibody, originally raised against TATA-binding protein (TBP), (a transcriptional activator). Normal huntingtin is localised in the cytoplasm but mutant huntingtin, in addition to being found in the cytoplasm is localised in the nucleus. Although polyglutamine repeat length seems to be one feature governing nuclear entry of huntingtin, another is the length of the whole molecule. Shorter fragments of huntingtin are translocated to the nucleus more

efficiently with longer fragments tending to form aggregates in the cytoplasm.[7] This finding is

further supported using antibodies directed at the N-terminus of huntingtin, which can detect the inclusions in the nucleus, but antibodies directed at internal epitopes of huntingtin cannot detect the inclusions. Thus, it seems that huntingtin within the inclusions is a truncated N-terminal fragment. Another possibility is that the full-length huntingtin protein is misfolded so that the

internal epitopes are sequestered.[3] Popular hypothesis is that the full-length mutant protein is cleaved by caspases (the apoptotic proteases) to yield the N-terminal fragment before

translocating to the nucleus.[6]

Mutant huntingtin can recruit transcription factors into the inclusion bodies. For example, recruitment of CREB-binding protein (CBP), (a transcriptional co-activator), and TBP into aggregates has been shown in vitro as well as in human HD post-mortem brains. CBP has been identified as a critical component of neuronal responses to neurotrophins. It is therefore possible that the sequestration of CBP results in a diminished response to trophic factors, which are

essential to neuronal survival.[8] In addition, mutant huntingtin can recruit normal huntingtin into insoluble aggregates both in vitro and in vivo, suggesting that an important pathogenic effect might lie in the sequestration of normal huntingtin resulting in seclusion of its functions. Wild-type huntingtin has a protective anti-apoptotic effect in cells. One report indicates that the anti-apoptotic effects of huntingtin occur via sequestration of HIP1, a pro-apoptotic molecule

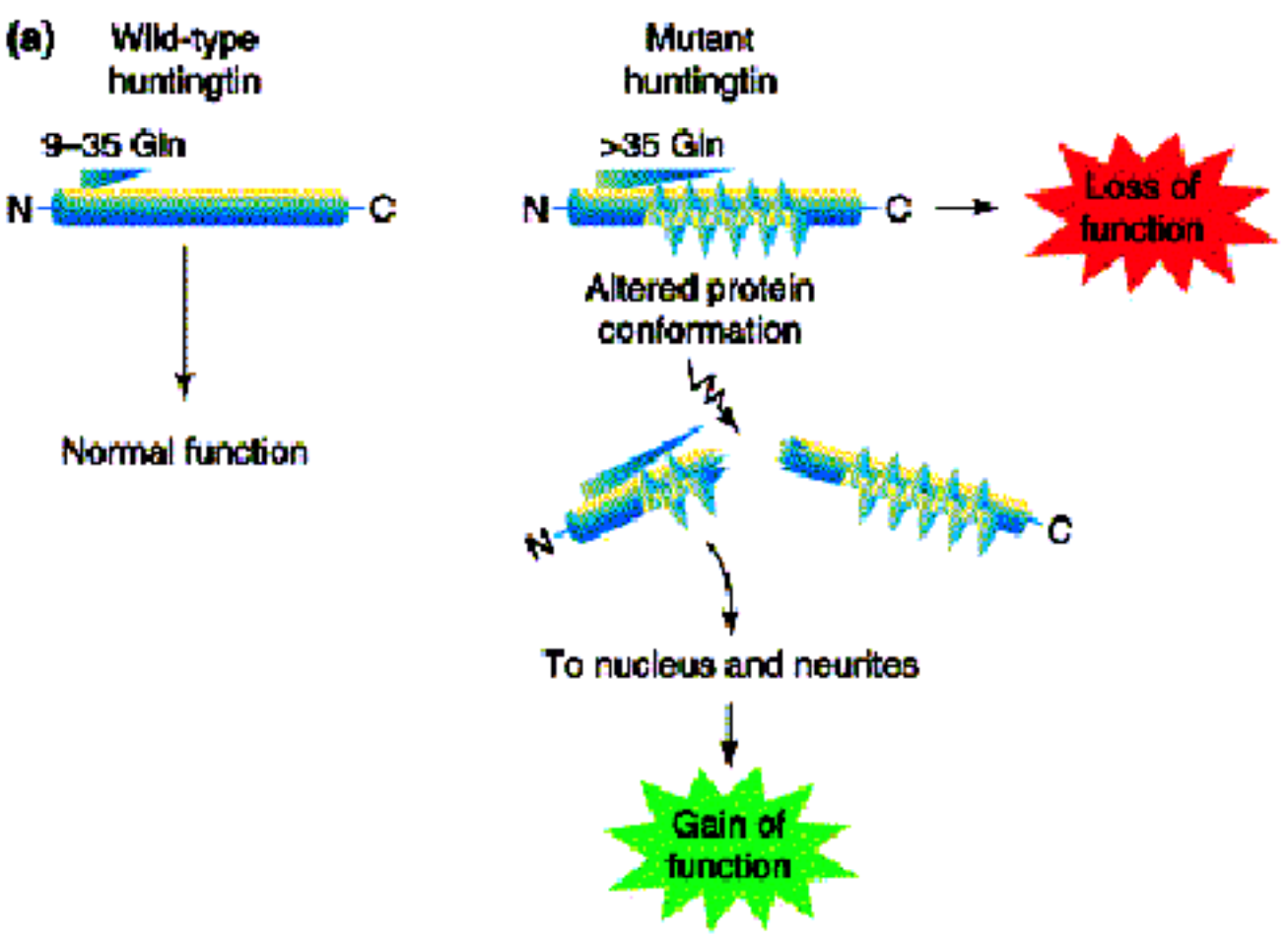
containing a novel-death effector domain.[6] It interacts efficiently with wild-type huntingtin but not with mutant huntingtin. Given the specific distribution of HIP1 in the brain, the researchers suggested that the inability of mutant huntingtin to modulate HIP1 contributes to the amplification cascade of cell death signals in HD. Thus, HD might be viewed as a double disease, that is, caused by both a new toxic property of the mutant protein and by a loss of the neuroprotective activity of normal huntingtin.

The huntingtin gene is expressed ubiquitously, not only throughout the brain but also in the peripheral tissues. But although peripheral abnormalities have been described, HD is primarily a disease of the CNS. The regional selectivity of the striatal medium spiny neurons to degeneration remains inadequately accounted for. The explanation could be that the genes whose transcription is altered by mutant huntingtin are especially relevant to neuronal function. Human HD and transgenic mouse models demonstrate downregulation of certain neurotransmitter receptor genes

that occur at the level of mRNA expression.[7] Therefore, it is possible that the mutant huntingtin exerts its toxic effects by affecting the expression of a set of genes that are important in neural and striatal functioning. The altered expression of this set of genes probably leads to the cellular dysfunction and eventual neuronal death that occurs in HD. In addition, it is thought that the neurotransmitter, dopamine, may have potentially toxic actions that contribute to the vulnerability

of the striatum.[9]

Figure 3. Potential mechanism of cell death in HD.



Processing of mutant huntingtin would generate an amino-terminal fragment that translocates into the nucleus and a carboxy-terminal portion that remains in the cytoplasm. Some full-length protein might also move into the nucleus, albeit with less affinity. The generation of amino-terminal fragments and inclusion bodies would coincide with increased toxic activity in cells. At the same time, (a) extension of the CAG repeat would cause a loss of function in the mutant protein and/or (b) the mutant protein could act negatively on the functions of the wild-type

protein.[6]

Mitochondrial dysfunction:

In cells expressing mutant huntingtin, mitochondria do not readily take up cationic dyes that

depend on intact charge gradients.[4] This data indicates that the mitochondrial membrane potential is impaired. 3-nitropropionic acid (3-NP) is an irreversible inhibitor of the enzyme, succinate dehydrogenase, which is found both in complex II of the mitochondrial respiratory chain and the citric acid cycle. In rats, systemic administration of 3-NP causes neurobehavioural and pathological abnormalities consistent with HD and in HD patients, the striatum has severe deficiencies in complexes II and III. In affected striatal and cerebral regions of the brain, glucose metabolism is decreased and precedes bulk tissue loss in HD patients. Taken together, these data point towards impairment in mitochondrial function as a contributing factor in HD.

THERAPIES

To alleviate symptoms:

The CARE-HD (Coenzyme Q10 and Ramacemide Evaluation in HD) is an NIH funded study involving 347 HD patients treated over a five-year period with a combination of Coenzyme Q10 (CoQ10) and Ramacemide hydrochloride (R). CoQ10 is an essential co-factor of the electron transport chain, as well as a potent free radical scavenger in lipid and mitochondrial membranes. R is a non-competitive, low affinity NMDA receptor antagonist that was successfully used in mouse models of epilepsy. In transgenic mouse models, high doses of dietary supplements of CoQ10/R enhanced motor performance, albeit transiently, and weight gain was evident compared to placebo controls. However, similar studies in humans to date did not achieve statistical

significance compared with placebo.[10]

In HD patients, magnetic resonance imaging confirms that creatine (a free radical scavenger, a

substrate for the enzyme creatine kinase and a precursor for ATP) is depleted.[4] Due to the possible role of energy dysregulation in pathogenesis, creatine was examined for possible neuroprotective effects in two transgenic mouse models of HD. The researchers found that creatine delays weight loss, improves motor performance, reduces the formation of intracellular

inclusions, delays striatal atrophy and significantly improves survival.[8] However, in human clinical trials efficacy has yet to be proven.

Caspases are cysteine proteases that play an important role in the execution of programmed cell death (apoptosis). It has been suggested that the aggregations of mutant huntingtin might induce

activation of important initiator caspases-8, -9, and -10.[11] Initiator caspases are responsible for cleavage and activation of downstream effector caspases, such as caspase-3. In cell-culture models, transcripts of huntingtin in which the caspase cleavage sites are mutated, demonstrate less nuclear localisation and show increased survival·[7] Caspase-3 cleavage of mutant huntingtin generates the small N-terminal fragment that forms nuclear inclusions. Such cells readily undergo apoptosis. Caspase inhibitors might provide protection by blocking a general cell death

pathway and/or by preventing the formation of the toxic N-terminal fragment.[4] Caspase inhibitors have been shown to exert neuroprotective effects and significantly improve survival in transgenic HD mice. One such inhibitor is minocycline, which is a derivative of the antibiotic tetracycline that crosses the blood-brain-barrier and inhibits caspases 1 and 3. Clinical trials are ensuing.

Potential cures:

Inhibition of protein expression of the mutant allele is one approach being taken. This inhibition is expected to be beneficial only if expression from the normal allele is preserved. Anti-sense oligonucleotides targeting the methionine initiation codon and exon 1 (the –25 to +35 region of the promotor) can inhibit expression of the stable incorporated green-fluorescent-huntingtin in a

cultured cell line to roughly half that of untreated cells·[4] Whether this is sufficient to rescue cell survival during long-term culture remains to be seen, and more importantly, whether it will be effective in targeting neural tissue in whole animals.

In animal HD models, behavioural signs analogous to HD can be improved by transplantation of embryonic striatal tissue into the degenerated striatum. In primates, striatal grafts have been shown to survive and improve motor function. In a report by Freeman et al. (2000), the post-mortem histological analysis of foetal striatal grafts implanted in the striatum of one patient who

died 18 months after implantation from reasons unrelated to surgery, were described.[12] The authors demonstrated that immature foetal striatal tissue could survive and differentiate into mature striatal tissue in the HD brain. Notably, the disease process did not appear to induce HD-like neurodegeneration within the cell implants and there was no evidence of immune rejection of

the cell implants by the host. These neuropathological results are timely because a French team, working in parallel, found in a pilot study that striatal grafts produce long-lasting motor, cognitive

and functional benefits in grafted HD patients.[13] Thus, striatal transplantation may be a viable treatment for HD.

CONCLUSION

For each new observation of the mechanism of HD, there is a possible therapeutic intervention. Thus, to broaden the therapeutic perspectives, it is important to identify all possible routes of disease manifestation. Many hypotheses exist today based on recent laboratory and clinical studies for the pathogenesis of HD, from mitochondrial dysfunction to loss of wild-type huntingtin function and to the deleterious functioning of mutant huntingtin. Thus, the rapid advances in understanding the pathogenesis, experimental therapeutics and neural transplantation predict a bright future for finding a cure for this devastating disease.

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