Caspases in Huntington's Disease

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Huntington's disease (HD) is an autosomal dominant condition, resulting from a mutation in huntingtin (htt). Htt is a novel protein, and its normal function is at present not well understood. Nuclear translocation of mutant htt in vitro up-regulates expression of the cell death gene caspase-1. We have demonstrated in a transgenic HD mouse model that caspase-1 and caspase-3 are transcriptionally up-regulated and activated. Underscoring the relevancy of these findings, recent results suggest that caspase-1 is activated in brains of humans with HD. Caspase activation results in the proteolytic cleavage of key cellular targets, including htt, leading to cell dysfunction. Caspase activation leading to cell dysfunction and death correlates with disease progression. In HD-transgenic mice, caspase inhibition resulted in a delayed onset of symptoms, a slowed progression, and prolonged survival. Caspase inhibition is a therapeutic strategy that merits evaluation in humans with HD. NEUROSCIENTIST 7(6):480–489, 2001

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In 1872, George Huntington, based on observations of families in Long Island, first described an inherited movement disorder that later became known as Huntington's disease (HD). Since then, much has been learned about HD pathophysiology and some of its many complexities have been revealed. Nevertheless, much remains to be learned to benefit patients with this disease, for which there is no present treatment.

HD is an autosomal dominant genetic condition characterized by progressive motor symptoms, cognitive deficits, and dementia. Onset can vary significantly from early childhood to late adult life. Most adult onset cases occur between 35 and 45 years of age and run their terminal course in 15 to 20 years. There is marked global atrophy of HD brains, yet the neuropathology demonstrates a specific pattern of neurodegeneration. The caudate nucleus and putamen undergo the most dramatic atrophy. It is the specific atrophy of the neostriatum, with coincident neuronal cell loss and astrogliosis, that contributes to the progressive motor symptoms. In the neostriatum, the medium-sized spiny neurons are most severely affected (Petersen and others 1999).

Huntingtin

In 1983, a collaborative effort linked HD to 4p16.3, and in 1993, the gene termed *IT15* was identified. *IT15*, a novel gene with no known homolog, contains a variable number of CAG repeats ranging from 6 to 180 within exon 1 (Gusella and others 1983; The Huntington's Disease Collaborative Research Group 1993). Asymptomatic individuals have 39 or fewer repeats, whereas adult onset HD patients usually have between 40 and 55. The number of repeats correlates with age of onset and disease severity (Andrew and others 1993; Snell and others 1993). *IT15* encodes a 348 kD protein termed huntingtin (htt). The normal function of htt is presently not well understood. Mice deficient in the murine homolog of the HD gene (*HDh*) fail to proceed to the formation of somites and organogenesis and die at embryonic day 8.5. Mice heterozygous for *HDh* have increased motor activity and cognitive deficits while demonstrating significant neuronal cell loss in the subthalamic nucleus (Duyao and others 1995; Nasir and others 1995; Zeitlin and others 1995). Htt interacts with an increasing number of cellular components including those involved in vesicle transport, endosomal-lysosomal organelles, transcription, and metabolism (Martin 1999; Petersen and others 1999; Kegel and others 2000).

Both wild-type (htt) and mutant (muhtt) proteins are widely expressed in many tissues in the body as well as diffusely throughout the brain (Strong and others 1993; Aronin and others 1995). Interestingly, the population of cells that are fated to die in HD, the striatal medium spiny neurons, expresses high levels of htt (Sharp and others 1995; Ferrante and others 1997). Htt is present in variable amounts in medium-sized striatal neurons, with few or none detectable in the majority of large striatal neurons (Ferrante and others 1997). Within neural tissue, htt expression is predominantly present in neuronal cells with little or no expression in glia (Gutekunst and others 1995). Most studies have found that neuronal wild-type htt is diffusely present in the cytoplasm and minimally in the nucleus or mitochondria (De Rooij and others 1996;

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Davies and others 1997). Within the cytoplasm of neurons, it is concentrated in the cell body, fibers, and nerve terminals (DiFiglia and others 1995; Sharp and others 1995; Trottier and others 1995).

Several transgenic mouse models of HD have been created. The R6/2 mice, the first HD model developed, contain a segment of exon 1 of the human HD gene containing a pathologic CAG repeat expansion. These mice demonstrate a progressive neurologic deterioration and characteristic neuropathologic abnormalities resembling HD (Mangiarini and others 1996). Mouse models with full-length mutant huntingtin have also been generated that demonstrate some neuropathologic features of HD but do not develop the rapid neurologic deterioration as seen in R6/2 mice (Reddy and others 1998; Hodgson and others 1999). Each one of the transgenic models has distinct and complementary strengths, adding to the tools available for the study of HD.

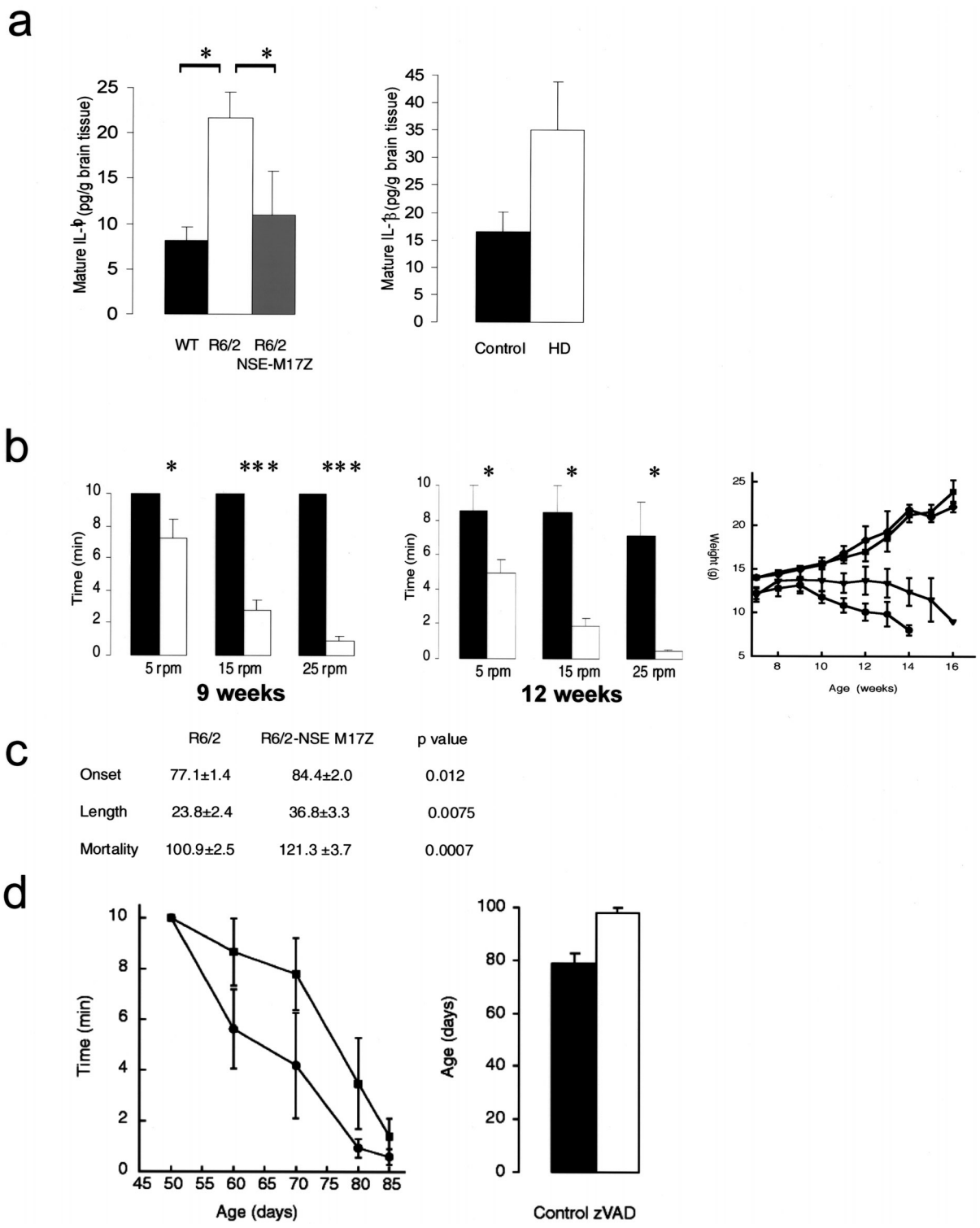
The caspase family, the Bcl-2 family, and the apoptotic protease-activating factor (Apaf-1) regulate mammalian apoptotic cell death (Yuan and Yankner 2000). Caspase-1 is a cysteine protease and the founding member of the mammalian caspase family. It is a homolog of the *Caenorhabditis elegans* death gene product CED-3 that, together with CED-4 (a homolog of Apaf-1), regulates apoptosis in the nematode (Yuan and others 1993). Caspase-1, also known as the interleukin-1β converting enzyme (ICE), has an important role mediating apoptosis in a variety of experimental paradigms (Friedlander 2000). Our laboratory has demonstrated a role of caspase-1-mediated neurodegeneration in animal models of amyotrophic lateral sclerosis (ALS) as well as acute neurologic insults such as cerebral ischemia, brain trauma, and spinal cord injury (Friedlander and others 1997; Hara and others 1997; Li, Ona, Chen, and others 2000; Li, Ona, Guegan, and others 2000; Sanchez Mejia and others 2001). Caspase-3, an effector caspase downstream of caspase-1, has also been implicated in neuronal cell death due to acute and chronic neurologic insults (Li, Ona, Chen, and others 2000; Li, Ona, Guegan, and others 2000; Sanchez Mejia and others 2001). Indeed, this recent work underscores the role caspase-1 and -3 play as important mediators of cell death in a variety of neurological conditions.

Apoptosis and Cell Dysfunction

In 1995, Portera-Cailliau and others were the first to demonstrate apoptotic cell death in human HD brains by detecting TUNEL-positive neurons and glia with occasional nonrandom DNA fragmentation in the neostriatum (Portera-Cailliau and others 1995). In R6/2 mice, Davies and others described nuclear membrane indentation and heterochromatin clumping as suggestive of the earliest stages of apoptotic cell death but failed to identify apoptotic nuclei in the neocortex or striatum (Davies and others 1997). There is debate concerning the extent to which apoptosis contributes to neuronal cell loss in HD. As we will describe, recent evidence strongly suggests that apoptotic pathways including the caspase family are clearly activated and play a key role in mediating cell dysfunction and cell death in HD and many other neurologic diseases.

Caspase-1 and caspase-3 activation in the brains of R6/2 mice provided the first in vivo evidence supporting a role for caspases in HD (Ona and others 1999; Chen and others 2000). This was further supported by findings in a neurotoxic mouse model of HD that demonstrated caspase-1 activation after intrastriatal malonate injections or systemic 3-nitropropionic acid administration (Andreassen and others 2000). Similar to R6/2 mice, we found evidence for caspase-1 activation in brain lysates from human HD patients (Ona and others 1999) (Fig. 1*a*). Caspase-1 and caspase-3 are transcriptionally up-regulated in a sequential fashion in R6/2 mice, with caspase-1 up-regulation observed as early as 7 weeks and caspase-3 up-regulation occurring at approximately 9 weeks (Chen and others 2000). A similar sequential activation of caspase-1 and caspase-3 is seen in a mouse model of ALS suggesting a possible characteristic pattern of caspase activation in chronic neurodegenerative diseases (Li, Ona, Guegan, and others 2000; Pasinelli and others 2000; Vukosavic and others 2000). There is a close temporal relation between caspase activation and disease onset and progression. Broad caspase inhibition with zVAD-fmk in an ALS mouse model resulted in decreased caspase-1 and caspase-3 gene transcription (Li, Ona, Guegan, and others 2000). The ability of a caspase enzymatic inhibitor to decrease caspase transcription, when caspases have no known direct function in gene transcription, suggests that caspase activation leads to the generation of intracellular and extracellular diffusible factors, such as IL-1β and reactive oxygen species, that act to induce further caspase activation within the cell and neighboring cells, which we refer to as "contagious apoptosis" (Fig. 2).

When caspase-1 is inhibited in R6/2 mice by crossbreeding with a mouse expressing a caspase-1 dominant negative transgene (M17Z) under the control of the neuron-specific enolase promoter, onset of motor symptoms is delayed, motor function is improved, and life span is extended (Ona and others 1999) (Fig. 1*b* and 1*c*). It is not known whether the dominant negative caspase-1 protein interacts with other caspases in addition to caspase-1 in this model. Administration of a broad caspase inhibitor (zVAD-fmk) also improved motor function and extended life span in R6/2 mice (Ona and others 1999) (Fig. 1*d*). Pharmacologic inhibition of both caspase-1 and caspase-3 is required to increase viability in R6/2 mice, suggesting that several caspases may act in concert in the progression of HD (Ona and others 1999; Chen and others 2000). Expression of the caspase-1 dominant negative transgene resulted in a delay in the appearance of neuronal intranuclear inclusions and astrogliosis, demonstrating an effect on the pathogenesis of the disease (Ona and others 1999) (Fig. 3*a-h*). These findings were the first to provide in vivo evidence of caspase involvement in HD and suggest that disease progression and symptoms might be ameliorated in humans with HD.



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	R6/2	R6/2-NSE M17Z	p value
Onset	77.1±1.4	84.4±2.0	0.012
Length	23.8±2.4	36.8±3.3	0.0075
Mortality	100.9±2.5	121.3 ±3.7	0.0007

Fig. 1. Caspase-1 activation in Huntington disease (HD). *a*, Caspase-1 is required for mature IL-1β production. IL-1β is a sensitive and specific marker for caspase-1 activation. Caspase-1 is activated in vivo in R6/2 mice and in human HD patients. Expression of a caspase-1 dominant negative inhibitor (R6/2-NSE M17Z) significantly decreases caspase-1 activity in R6/2 mice. *b*, Inhibition of caspase-1 in R6/2-NSE M17Z mice (black bars) results in improved motor performance at 9 and 12 weeks at various speeds on the rotarod when compared with R6/2 mice (white bars). Expression of the caspase-1 dominant negative transgene decreases the observed weight loss in R6/2 mice (squares, wild type [WT]; diamonds, NSE M17Z; circles, R6/2; and triangles, R6/2-NSE M17Z). $^{*}P < 0.05$; $^{***}P < 0.0005$. *c*, Disease onset and mortality were delayed in R6/2-NSE M17Z mice compared with R6/2 controls. *d*, Motor symptoms (as measured by rotarod performance) were improved and mortality was delayed in R6/2 mice treated with a broad caspase inhibitor (zVAD-fmk) compared with R6/2 mice given a control compound. (Reprinted by permission from Nature 399:263-267. Copyright 1999 Macmillan Magazines Ltd.)

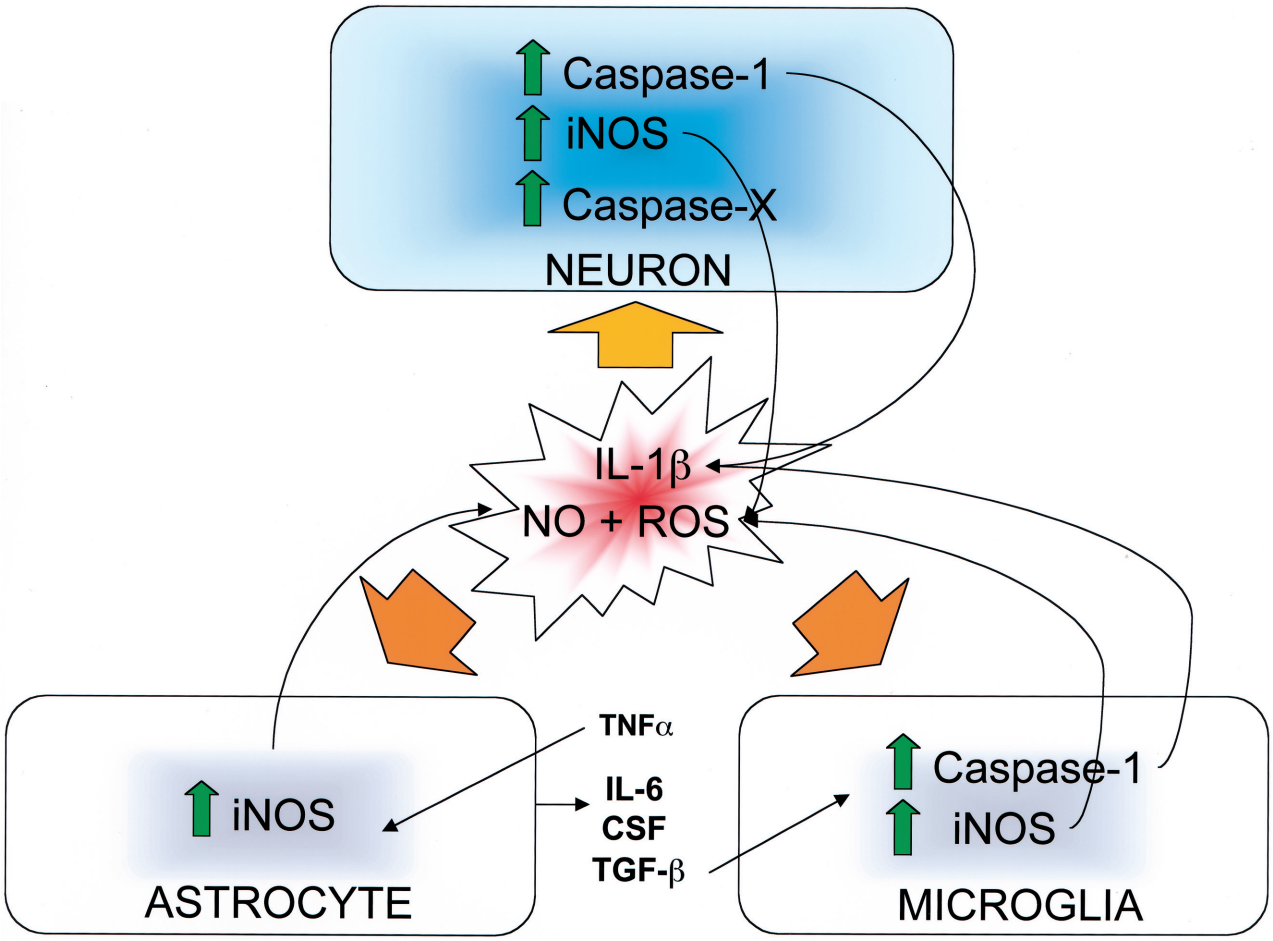
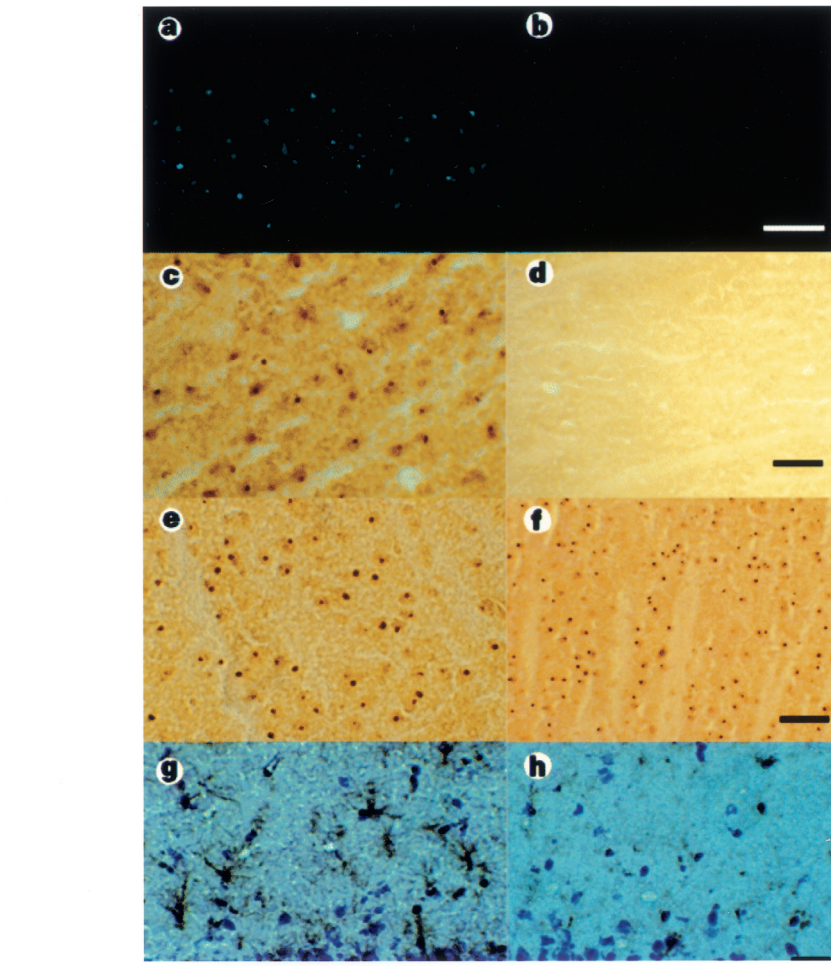


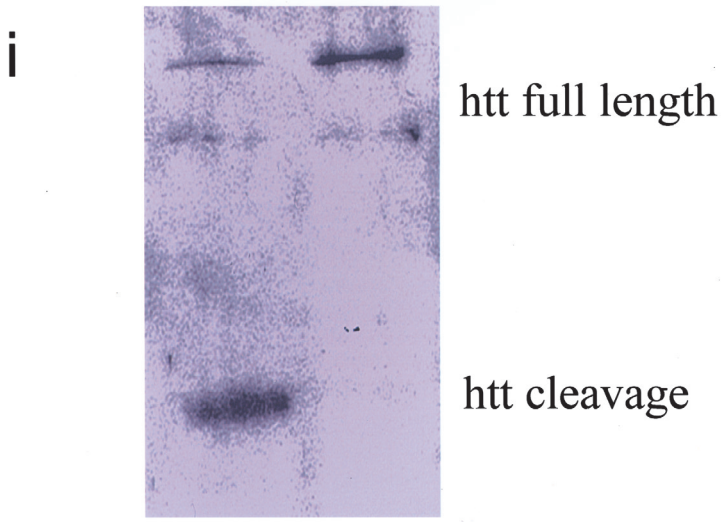
Fig. 2. Contagious apoptosis. Translocation of mutant huntingtin to the nucleus has been shown to up-regulate caspase-1. Caspase-1 activation leads to the production of proinflammatory and proapoptotic mature interleukin-1β (IL-1β). Secreted mature IL-1β induces IL-1β, nitric oxide (NO), and reactive oxygen species (ROS) production in neighboring astrocytes and microglia. These secreted proinflammatory and proapoptotic mediators then induce further caspase and the inducible form of nitric oxide synthase (iNOS) activation in neurons generating a detrimental self-perpetuating cycle leading to cell dysfunction and cell death. Other cytokines, which are stimulated by IL-1β and NO, including TNF-α, IL-6, CSF, and TGF-β, may also play a role in the pathogenesis of Huntington's disease.

Minocycline, a tetracycline derivative, was found to have anti-apoptotic and anti-inflammatory properties by down-regulating caspase-1 and the inducible form of nitric oxide synthase (iNOS) in a rat model of cerebral ischemia (Yrjanheikki and others 1999). Based on this information and the similarities in the mechanisms of cell death in ischemia and HD (i.e., caspase-1 activation and iNOS up-regulation), minocycline was tested in R6/2 mice (Ona and others 1999; Tabrizi and others 1999). Minocycline decreased expression of caspase-1 and caspase-3, resulting in delayed disease onset, improved motor function, and extended viability compared with saline-treated mice (Chen and others 2000) (Fig. 4*a-c*). iNOS is up-regulated in mouse models of HD as well as human HD patients (Tabrizi and others 1999; Chen and others 2000) (Fig. 4*d*). Minocycline treatment significantly decreased iNOS activity in R6/2 mice (Fig. 4*e*).

The above findings in mouse models of HD have been supported with in vitro studies (Saudou and others 1998; Li, Ona, Geugan, and others 2000; Rigamonti and others 2000). In vitro studies have shown that cells expressing mutant htt undergo apoptosis and that an N-terminal cleavage fragment of htt (discussed below) contributes to this toxicity partly by inducing caspase-1 expression (Saudou and others 1998; Li, Ona, Guegan, and others 2000). Other caspases, both upstream and downstream of caspase-1 and caspase-3, may also play an important role in HD (Yuan and Yankner 2000). The improved clinical features in R6/2 mice resulting from caspase-1 and caspase-3 inhibition underscore their important role in HD pathogenesis. Indeed, caspase inhibitors and minocycline provide hope for their utility as novel therapeutic approaches for the treatment of HD in humans. Based on these results and the long safety record of minocycline, human clinical trials are currently in preparation.

In addition to inducing apoptosis, caspase activation in the context of HD may result in cell dysfunction. Abnormal neurotransmitter receptor down-regulation is detected in R6/2 mice, likely contributing to the HD-like neurologic dysfunction (Cha and others 1998). Expression of the caspase-1 dominant negative inhibitor





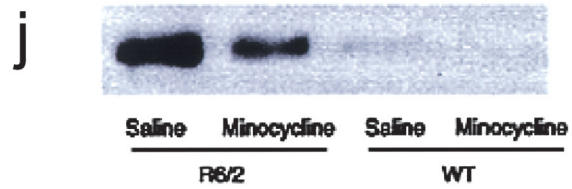
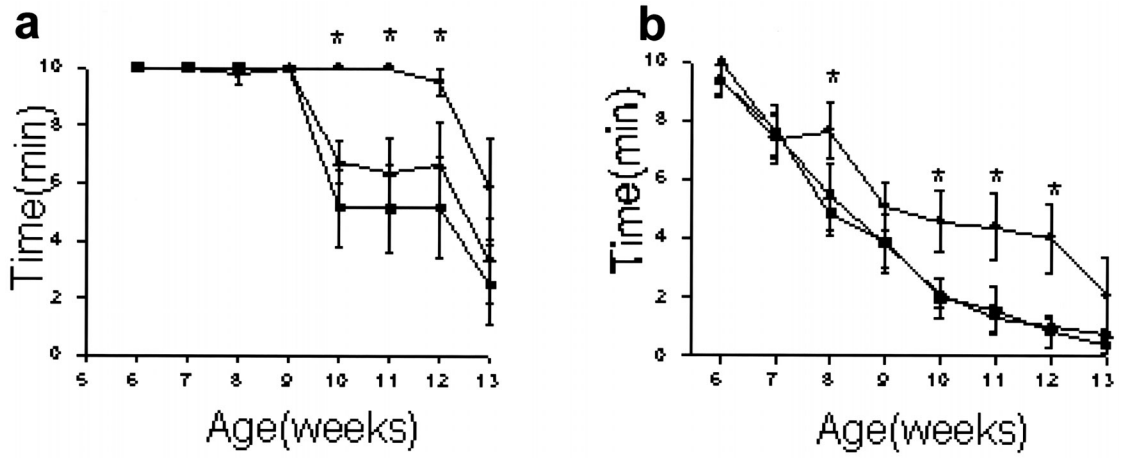


Fig. 3. Caspase-1 inhibition decreases huntingtin (htt) cleavage and inclusion formation. At 9 weeks, R6/2 mice demonstrate abundant ubiquitin-positive neuronal intranuclear inclusions (*a* and *c*). Neuronal intranuclear inclusions are not detected in age-matched R6/2-NSE M17Z mice (*b* and *d*). However, by 12 weeks of age, neuronal intranuclear inclusions are detected in both R6/2 and R6/2-NSE M17Z mice, demonstrating a delay in inclusion formation by expression of the M17Z transgene. At 12 weeks, the ubiquitin-positive nuclear inclusions are smaller in R6/2-NSE M17Z mice (*f*) when compared with R6/2 mice (*e*). Astrogliosis is also decreased in R6/2-NSE M17Z (*h*) mice compared with R6/2 mice (*g*). *i*, Cleavage of full-length endogenous htt is increased in R6/2 mice, generating increased levels of htt cleavage fragments (*left lane*) and depletion of full-length wild-type (WT) htt. Htt cleavage is inhibited in R6/2-NSE M17Z mice (*right lane*). *j*, Increased endogenous wild-type htt cleavage in R6/2 mice compared with wild-type mice is also inhibited in R6/2 mice treated with minocycline. The decreased cleavage of endogenous htt is presumably due to caspase inhibition or down-regulation. Scale bar, 50 μm (*a-b*); 40 μm (*c-h*). (Reprinted by permission from Nature 399:263-267 copyright 1999 Macmillan Magazines Ltd.)



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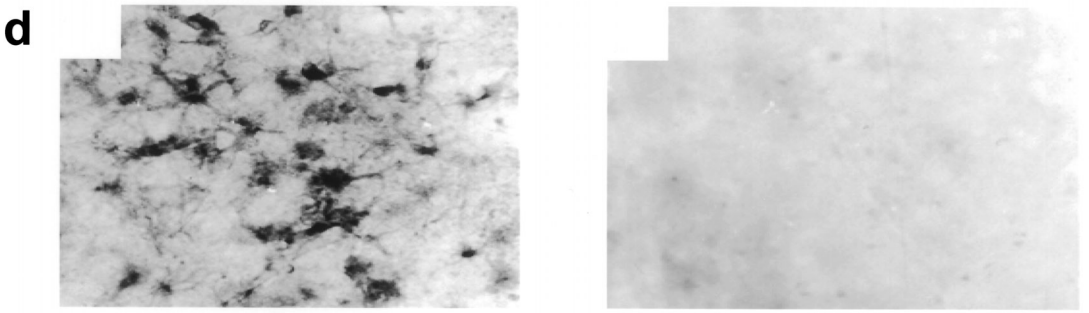
	Saline	Minocycline	Tetracycline
onset	69.6±3.3	76.4±4.5	72.2±2.1
P value		0.28	0.52
mortality	86.1±3.7	97.7±3.0	91.0±2.6
P value		0.032	0.30



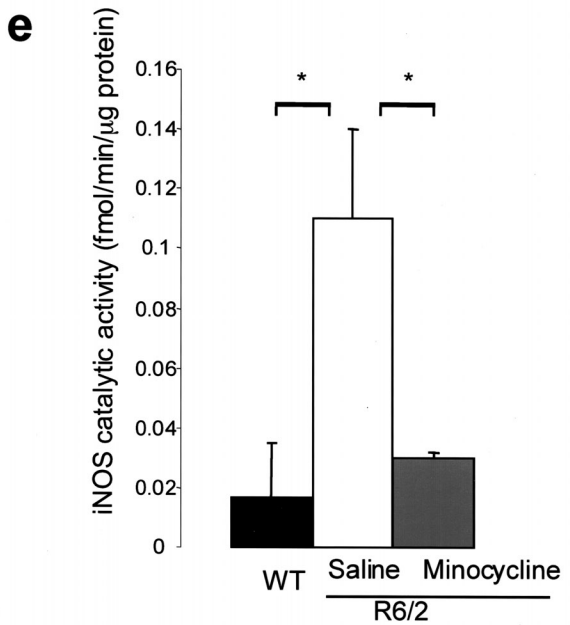


Fig. 4. Caspase-1 and caspase-3 inhibition with minocycline improves clinical phenotype of R6/2 mice. Caspase-1 and caspase-3 inhibition with minocycline improved motor performance on rotarod in R6/2 mice at 5 (*a*) and 15 (*b*) rpm (circles, minocycline; triangles, tetracycline; and squares, saline). Tetracycline was used as a control because it does not effectively cross the blood-brain barrier. *$P < 0.05$. *c*, Disease onset and mortality were delayed in R6/2 mice administered minocycline when compared with R6/2 mice receiving saline. *d*, The inducible form of nitric oxide synthase (iNOS) expression is increased in the caudate nucleus of Huntington's disease patients (*left*) compared with control patients (*right*). *e*, iNOS activity, as measured by nitric oxide production, is up-regulated in R6/2 mice compared with control mice. Minocycline treatment inhibits this up-regulation in R6/2 mice. WT = wild type. (Reprinted by permission from Nature Med 6:797:801 copyright 2000 MacMillan Magazines Ltd.)

delays the onset of neuroreceptor down-regulation (Ona and others 1999). It remains to be determined if caspase inhibition would also ameliorate other forms of cell dysfunction. Unlike in acute cell death, in chronic neurodegenerative diseases, caspase activation within a cell appears to take place over a long period of time (weeks to months or longer) and suggests that caspases may contribute to cell dysfunction in addition to their role in apoptotic cell death. Since neuronal dysfunction manifests itself as neurological dysfunction, caspase inhibition may theoretically improve clinical symptoms as well as slow the progression of the disease.

Huntingtin Cleavage

Caspase-1 and -3 can efficiently cleave huntingtin, producing several fragments (Goldberg and others 1996; Wellington and others 1998). In humans, wild-type and mutant htt undergo distinct proteolysis in the cortex and striatum. Cleavage of wild-type and mutant htt in the cortex occurs in two regions producing three fragments in equal amounts. In contrast, in the striatum three different-sized fragments are formed, with a 20 to 50 kD N-terminal fragment being increased in HD patients relative to controls (Mende-Mueller and others 2001). In humans, amino-terminal fragments with a normal number of CAG repeats are diffusely present in the cytoplasm, whereas fragments with expanded polyglutamine repeats are present in the cytoplasm and nucleus where they can concentrate in dystrophic neurites and intranuclear inclusions, respectively (DiFiglia and others 1997). Full-length mutant or wild-type htt, on the other hand, appear to remain in the cytoplasm (DiFiglia and others 1997; Hackam and others 1998; Saudou and others 1998). The elevated N-terminal fragment observed in the striatum of HD patients may be the fragment contributing to nuclear and cytoplasmic protein aggregation, caspase activation, and cellular toxicity. Hence, caspase activation and subsequent htt cleavage appear to contribute to presence of the classic neuropathologic markers of HD and to the coincident neuronal cell dysfunction and death (Fig. 5).

Whether intranuclear inclusions themselves are harmful or beneficial to cells is debated (Davies and others 1997; DiFiglia and others 1997). Intranuclear inclusions have been shown to correlate with cell death in vitro and with disease progression in humans and R6/2 mice (Davies and others 1997; DiFiglia and others 1997; Ona and others 1999; Ferrante and others 2000). Nevertheless, some studies have argued against a direct association between inclusions and cell death. Blocking nuclear localization of muhtt in vitro suppressed its ability to form intranuclear inclusions and to induce neurodegeneration, yet the presence of inclusions did not correlate with cell death (Saudou and others 1998). Furthermore, broad caspase inhibition with zVAD-fmk in cells transfected with muhtt improved viability without affecting the amount of intranuclear and cytoplasmic inclusions, whereas zDEVD (a caspase-3-specific inhibitor) decreased inclusions without affecting viability, suggesting that inclusions are not directly responsible for cell death (Kim and others 1999). Although these findings need to be reconciled, at present it is clear that translocation of the muhtt N-terminal fragment to the nucleus is sufficient to contribute to cell dysfunction and death (Cooper and others 1998; Saudou and others 1998). It is not clear whether a free N-terminal fragment of muhtt, the formation of inclusions, or both contribute to the observed toxicity. Whether mutant htt serves to activate the caspase cell death pathway or other triggers initiate caspase activation leading to the cleavage of mutant htt is unknown, but both of these processes, once initiated, likely serve to form a vicious detrimental cycle (Fig. 5).

Interestingly, wild-type htt was found to protect cells in vitro from apoptotic stimuli including serum withdrawal, death receptor activation, and pro-apoptotic Bcl-2 homologs (Rigamonti and others 2000). We have found that R6/2 mice have increased endogenous wild-type htt cleavage products, as well as depletion of the full-length protein compared with control mice, presumably from caspase activation (Figure 3*i-j*). This suggests that some of the detrimental effects that contribute to cell dysfunction and death may be due to diminished protection from decreased levels of full-length endogenous wild-type htt (Chen and others 2000). Caspase inhibition with minocycline or by expression of the caspase-1 dominant negative transgene in R6/2 mice resulted in decreased full-length htt cleavage (Figure 3*i-j*). In vitro studies demonstrated that caspase-3 activation is dependent on the presence of muhtt and that the protective effect of wild-type htt is likely upstream of caspase-3 (Rigamonti and others 2000). The ability of muhtt to induce caspase-1 activation and of wild-type htt to inhibit caspase-3 activation and apoptosis suggests that caspase-1 may be acting upstream of caspse-3. This hypothesis is supported by the sequential activation seen in our in vivo studies in the R6/2 mouse, although R6/2 mice do not require htt cleavage to form the N-terminal fragment (Ona and others 1999).

Interestingly, in a mouse model, expression of mutant full-length human htt in the absence of wild-type htt results in testicular atrophy and apoptosis. In this model, expression of wild-type htt protected from testicular cell death, providing in vivo evidence for a protective role of wild-type htt in mutant htt-mediated toxicity (Leavitt and others 2001). Furthermore, in vivo neuronal inactivation of htt in an adult conditional knockout mouse results in a progressive neurodegenerative phenotype supporting its role in neuronal cell survival (Dragatsis and others 2000). It remains to be determined whether this protection could be extended to neuronal cells in an HD mouse model that features symptoms similar to human HD patients, such as R6/2 mice. It would also be interesting to determine whether mutant htt has any effect on other mediators of cell dysfunction and cell death.

Inflammation

Although extensive research into the role of inflammation in HD remains to be done, it appears that inflam-

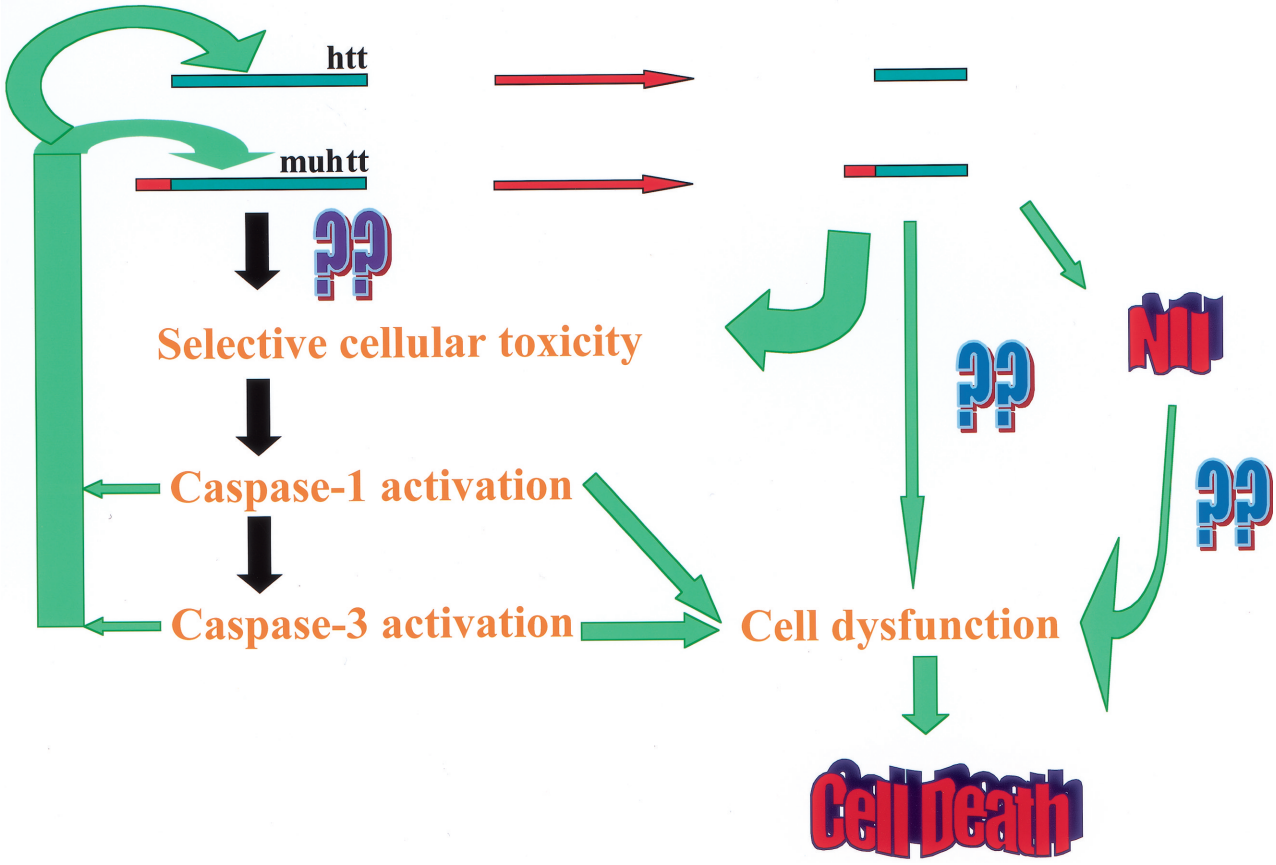


Fig. 5. Cellular pathogenesis of Hunington's disease. Expression of mutant huntingtin (muhtt) initiates a toxic sequence of events culminating in caspase up-regulation and activation. The cause for the vulnerability of certain cell types is not well understood. According to this model, a certain level of "cell toxicity" is reached, casapse-1 is activated, and it thereafter may directly or indirectly activate caspase-3. Both of these caspases then cleave the full-length htt molecule generating the toxic fragment (muhtt fragment). As a detrimental feedback loop, muhtt fragments exacerbate cell toxicity and accelerate the formation of neuronal intranuclear inclusions (NIIs) causing further caspase activation. Progression of cell dysfunction (in part manifested as a decreased number of specific neuroreceptors) and cell death translate to symptomatic progression.

matory responses are involved in HD. Increased iNOS and caspase-1 expression have been observed in R6/2 brains, which lead to the production of the proinflammatory molecules nitric oxide (NO) and mature interleukin-1β (IL-1β) (Ona and others 1999) (Fig. 1*a* and 4*d-e*). Proinflammatory NO and IL-1β likely attract microglia and contribute to the observed astrogliosis while inducing proapoptotic pathways in neighboring cells. NO production can lead to the generation of reactive oxygen species that can further mediate the induction of apoptotic pathways in neighboring cells.

Other Mechanisms

It is clear that caspases play an essential role in HD neurodegeneration, but there are many other mechanisms that may contribute to cellular dysfunction and cell death in HD. Muhtt has been implicated in several mechanisms of cell dysfunction including excitotoxic damage, mitochondrial dysfunction, transcriptional deregulation, metabolic deregulation, free-radical damage, and autophagy (Petersen and others 1999; Kegel and others 2000). Some of these mechanisms can trigger caspase activation and induce apoptosis. Whether they act in parallel or in concert, upstream or downstream, of caspase activation is now being intensely investigated. These experiments will begin to fill some of the gaps in knowledge, connecting all the sites of mutant htt action into a general picture of cell dysfunction and death.

Future Directions

Huntington's disease remains an untreatable condition, yet as research continues, the information learned is slowly leading to a practical understanding of the mechanisms of pathogenesis. Already this understanding has lead to some promising prospective treatments. In fact, minocycline is entering clinical trials in the near future for HD patients. Nevertheless, more work elucidating the molecular pathways involved in HD and how they interact is needed to develop additional targets for pharmacologic intervention and raise the prospect of ameliorating or curing Huntington's disease.

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