FOCUS: PSYCHIATRY AND PSYCHOLOGY

Huntington's Disease: The Past, Present, and Future Search for Disease Modifiers

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Huntington's disease (HD†) is an autosomal dominant genetic disorder that specifically causes neurodegeneration of striatal neurons, resulting in a triad of symptoms that includes emotional, cognitive, and motor disturbances. The HD mutation causes a polyglutamine repeat expansion within the N-terminal of the huntingtin (Htt) protein. This expansion causes aggregate formation within the cytosol and nucleus due to the presence of misfolded mutant Htt, as well as altered interactions with Htt's multiple binding partners, and changes in post-translational Htt modifications. The present review charts efforts toward a therapy that delays age of onset or slows symptom progression in patients affected by HD, as there is currently no effective treatment. Although silencing Htt expression appears promising as a disease modifying treatment, it should be attempted with caution in light of Htt's essential roles in neural maintenance and development. Other therapeutic targets include those that boost aggregate dissolution, target excitotoxicity and metabolic issues, and supplement growth factors.

THE CLINICAL PRESENTATION OF HUNTINGTON'S DISEASE

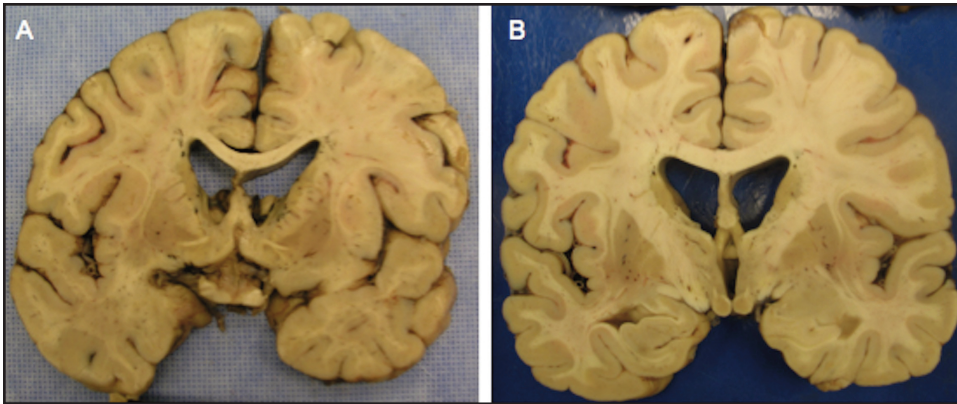Huntington's disease (HD) is a fatal neurodegenerative disorder affecting five to eight per 100,000 persons of European descent [1]. In 1872, a 22-year-old American neurologist published the first complete description of the disease [2]. George Huntington accurately characterized HD as a genetic condition and described the clinical presentation of HD as a triad of motor, emotional, and cognitive disturbances. The hallmark symptom of HD is the presence

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Figure 1. Striatal pathology in Huntington's disease. Huntington's disease (HD) causes overall cortical atrophy and selective cell death of the medium spiny neurons in the striatum, with corresponding enlargement of the lateral ventrical, as evidenced by coronal autopsy sections from a 54-year-old male HD patient (a) and a 47-year-old female HD patient (b).

of involuntary movements, called chorea [3]. Symptom onset typically occurs in midlife and the disease progresses over the next 15 to 20 years [4].

In George Huntington's day, diagnosis of HD could be tricky, relying heavily upon family history, but also upon postmortem brain analysis, where several pathological features are observed in Huntington's diseased brains. There is overall atrophy, with marked cell loss in the striatum (caudate/putamen), and globus pallidus, with corresponding ventricular enlargement and gliosis [4] (Figure 1). Cortical pyramidal neuron degeneration also occurs, particularly in association areas of the temporal, frontal, and parietal lobes [5]. Within the HD striatum, the selective progressive loss of GABA-ergic projection neurons (called medium spiny neurons) results in choreic symptoms [6]. The medium spiny neurons receive robust cortical glutamatergic inputs [7], implicating excitotoxicity — a calcium-mediated pathological process that damages or kills cells by overstimulation of glutamate receptors — in the selective neuronal death seen in HD.

Back in 1872, George Huntington was able to categorize HD as a genetic condition. We now know that HD is an autosomal dominant disease, meaning that each offspring of an affected individual has a 50 percent chance of inheriting the disease, the disease does not skip a generation, and males and females are equally at risk [8]. One mutated gene is sufficient to cause the disease, regardless of the presence of a normal gene inherited from the other parent [9], and, in fact, homozygous individuals do not appear to differ significantly from heterozygotes in terms of age of onset or symptom severity [10].

THE MOLECULAR BIOLOGY OF THE *HD* GENE

The effort to find the *HD* gene is a remarkable story of collaboration between many researchers amid the earliest efforts of gene sequencing and cloning. Using genetic markers to probe specific American and Venezuelan kindreds, the gene responsible for the HD mutation was mapped to the tip of chromosome 4 [11]. By 1993, the Huntington's Disease Collaborative Research Group isolated the HD mutation to a large gene (*IT15*, also called the *HD* gene) that encoded a 348 kDa novel protein [12]. The protein product, termed huntingtin (Htt), is the sole product expressed by this gene sequence. Expansion of a normally occurring glutamine (CAG) repeat within the Htt protein results in an extended N-terminal domain [13]. The average size of CAG repeats is 16 to 20 in the normal population and >36 in the affected population [14]. Htt glutamine repeat lengths between 27-35 are in the
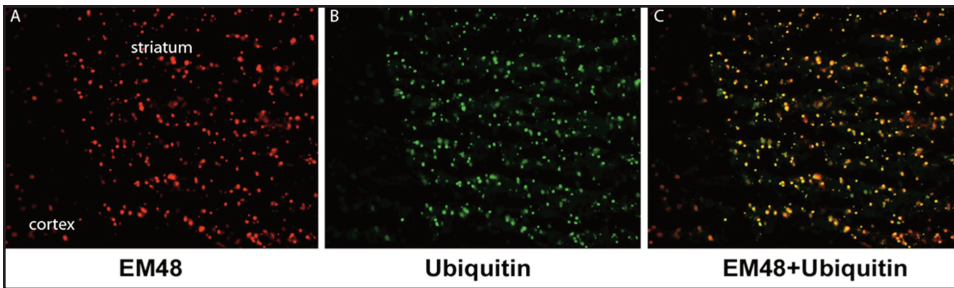
Figure 2. Aggregate formation in a 140 polyQ knock-in mouse model of HD. In a model of HD made by inserting a chimeric mouse/human exon 1 with 140 CAG repeats into mice, inclusions and aggregates can be seen in the striatum and cortex using the EM48 antibody (a), which is specific for the N-terminal region of the mutant huntingtin protein. These bodies also contain ubiquitin (b), with colocalization of the two proteins (c).

high normal range, but may elongate in future generations due to the unstable nature of the expansion; polyglutamine (polyQ) repeat lengths between 36 and 39 result in reduced penetrance, with delayed or no symptom onset [15].

The fact that HD is an inherited mutation with an expanded CAG repeat in the coding region of a gene lumps it into a category with eight more otherwise unrelated disorders, including dentatorubropallidoluysian atrophy (DRPLA), spinobulbar muscular atrophy (Kennedy's disease), and several spinocerebellar ataxias, including type-1 (SCA1) and type-3 (SCA3 or Machado-Joseph disease) [16]. In these disorders, mutant alleles encode a protein with a corresponding number of polyQ repeats [13]. Each of these triplet repeat disorders demonstrate a progressive neurological phenotype in specific brain regions. The age of disease onset is inversely proportional to the number of CAG repeats — the longer the polyQ stretch, the earlier the individual will experience symptoms.

The dominant pattern of heredity displayed by HD focused immediate research efforts on a gain-of-function model. Researchers thought that Htt would be preferentially expressed in the areas most severely affected in HD, namely the striatum and cortex. But Htt is highly expressed in the entire brain and testis, predominately in neurons, as well as in glial cells [17]. Within the cell, Htt is a mostly cytoplasmic protein that is also found at low levels in the nucleus [18].

Once the *HD* gene was isolated, researchers were able to clone it and insert a mutated form into animals. Animal models catapulted the HD field forward. Htt is a highly conserved protein, and models of HD have been constructed in animals as diverse as *C. elegans, D. melanogaster*, and zebrafish [19] (Table 1). In 1995, targeted Htt disruption confirmed a gain-of-function model in HD [20-22], and in 1996, researchers in Gillian Bates' lab showed that expression of an expanded Htt exon 1 alone was sufficient to induce a progressive neurological phenotype in mice [23].

Expression of mutant Htt in the animal models revealed a distinctive cellular phenotype — intranuclear inclusions and cytoplasmic aggregates that were mirrored in HD human patients [24] (Figure 2A). Aggregation was found in many brain areas and therefore could not explain the vulnerability of the striatum. The aggregates in dystrophic neurites were found in presymptomatic patients; however, the presence of intranuclear inclusions appeared to coincide with the onset of HD symptoms [25].

A theory about HD pathogenesis emerged, strongly based on the data gleaned from the animal models. Functional subunits of the proteosome, ubiquitin, and heat shock proteins [24,26,27] are localized to polyQ disease inclusions, suggesting a cellular clearance effort (Figure 2B and 2C). If mutant Htt is resistant to proteolysis, then protein turnover is delayed. The concentration of protein increases with time, leading to ag-

Table 1. Animal Models of HD.

Model	Construct/ Promoter	PolyQ Length	Age of Onset	Pathology	Behavioral Phenotype	Year
Mouse R6/1	Human Htt promoter; ~1.9kb fragment of 5′ human HD gene	115	5 m	Intranuclear and neuropil aggregates throughout the brain; global brain atrophy; minimal cell death	Tremors and gait abnormalities; rotarod deficit; clasping behavior; learning deficit	1996 [23]
R6/2		144	2 m			
Drosophila	GAL4-UAS system-using eye-specific P element expression vector pGMR; human *HD* exon 1	75 or 120	2 or 10 days	Late-onset progressive neurodegeneration dependent on repeat length; nuclear accumulation but no inclusions	Expression restricted to eyes	1998 [19]
Zebrafish	Expanded N-terminal fragment of Htt protein fused with GFP	102	24 h post fertilization	Increase in apoptotic cells, inclusions in non-apoptotic cells	Increase in embryonic lethality or in embryos with abnormal morphology	1998 [134]
Mouse N171-82Q	N-terminal 171 amino acids of human Htt; mouse prion promoter	82	5 m	Inclusions in striatum, cortex, hippocampus and amygdala; striatal degeneration	Tremors and gait abnormalities; rotarod deficit; loss of coordination; hypokinesis	1999 [135]
Conditional mouse	TetO regulatable; Chimeric mouse/human exon 1. Replace the endogenous	94	4.5 m	Nuclear/cytoplasmic aggregates in striatum, cortex, and hippocampus; striatal degeneration; gliosis	Clasping behavior, tremor, decreased grooming	2000 [60]
Mouse Hdh Q150	polyQ with expanded polyQ; mouse Hdh promoter	150	4 m	Nuclear inclusions in striatum; striatal gliosis	Clasping behavior; gait abnormalities; rotarod deficit; hypoactivity	2001 [136]
Mouse YAC 128	YAC expressing full-length human Htt; human HD promoter	128	3 m	Inclusions in striatum; neuron loss in striatum.	Rotarod deficit; clasping; gait abnormalities; circling behavior	2003 [137]
Mouse *Hdh* Q140	Replace mouse Htt exon 1 with expanded chimeric mouse/ human exon 1; mouse Hdh promoter	140	12 m	Nuclear and neuropil inclusions in striatum, cortex, nucleus accumbens, and olfactory tubercle	Increased locomotor activity and rearing at 1 month, followed by hypoactivity and gait abnormalities	2003 [138]
Transgenic Rat	A truncated Htt fragment; endogenous rat promoter	51	Adult onset	Neurological phenotypes, intracellular inclusions, striatal shrinkage	Progressive motor dysfunction	2003 [139]
Mouse BAC-HD	Full-length human Htt; human *HD* promoter	97	3 m	Synaptic dysfunction; cortical and striatal atrophy	Rotarod deficit	2008 [140]
Rhesus Macaque Monkey	Human HD exon 1 fused to GFP; Human polyubiquitin-C promoter	84	Birth to 1 week	Neuronal inclusions	Dystonia, chorea	2008 [141]
Rat BACHD	Human full-length HD genomic sequence; human HD promoter	97	Early onset	Cortical and striatal aggregates; neuropil aggregates appear earlier than inclusions; reduced dopamine receptor binding was detectable by in vivo imaging	Robust, early onset and progressive motor deficits and anxiety-related symptoms	2012 [142]

HD, human huntingtin gene; *Hdh*, mouse huntingtin gene; m, months of age; GAL4-UAS system, Transgenic flies expressing GAL4, a yeast transcriptional activator, are crossed with UAS-transgenic flies, carrying a gene of interest inserted downstream of the UAS (upstream activating sequence); YAC, yeast artificial chromosome; BAC, bacteria artificial chromosome; GFP, green fluorescent protein
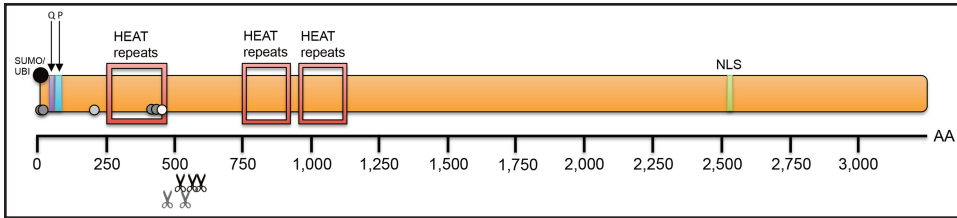
Figure 3. Huntingtin Protein Domains (adapted from Zuccato 2010). Huntingtin (*Htt*) is a large protein (352 KDa) with several highly conserved protein domains. The N-terminus contains the polyglutamine (polyQ) stretch that causes Huntington's disease when elongated, as well as a proline rich region (PRR). There are three sets of HEAT repeats (*H*tt, elongation factor 3, the PR65*/A* subunit of protein phosphatase 2A and the lipid kinase *T*or) that stretch throughout a large portion of the protein. Several cleavage sites have been identified in *Htt*, concentrated between amino acids 400-600, as well as a nuclear localization signal (NLS) toward the C-terminus. *Htt* also is subject to multiple forms of post-translational modifications, including acetylation, phosphorylation, palmitoylation, sumoylation and ubiquitination, which can be altered in the presence of the expanded allele.

gregation. The aggregates draw other proteins in (including normal Htt), sequestering them and rendering them useless [28]. Key cellular components, such as neurofilaments, are disrupted by aggregate formation [29]. The cell then becomes dysfunctional, dies, and the patient becomes symptomatic.

PHYSIOLOGICAL MODIFIERS

Due to its large size, the tertiary structure of Htt remains unknown, but the structure of several of its protein domains has been described. Analysis of Htt protein composition revealed a glutamine-rich region followed by a proline-rich domain, several caspase cleavage sites, and three sets of HEAT repeats (*H*tt, *e*longation factor 3, the PR65*/A* subunit of protein phosphatase 2A and the lipid kinase *T*or) (Figure 3) [30-32]. The polyQ stretch, being the subject of intense speculation, is not found in all organisms that express an Htt homolog. The polyQ stretch is absent in the N-terminal in *Drosophila* and *Ciona* (sea squirt), maintained at 4 glutamines in fish, birds, and amphibians, and expanded to its longest stretch in humans [33]. Deletion of Htt's polyQ stretch in mice causes neurological consequences and alterations in energy homeostasis in adults, but its absence does not appear to overtly impact development [34]. Loss of the entire Htt protein results in embryonic

lethality in mice due to organization defects in the extra-embryonic tissues [22,35].

The Htt proline-rich region is found only in mammals [33], and although it may contribute to the solubility of the protein [36], deletion of the proline-rich domain in mice does not appear to significantly affect Htt's normal function [37]. In contrast, the large majority of the HEAT repeats are present throughout all the homologs, including insects [33]. HEAT repeat proteins are typically very large, function as part of protein complexes, and are often involved in cytoplasmic transport processes [30]. The conserved nature of the HEAT repeats in Htt is perhaps our best clue to Htt's normal function, as their presence indicates a propensity to interact with other proteins, and suggests Htt is a type of scaffold on which other proteins can assemble.

The presence of accumulated misfolded proteins classifies HD as a conformational disease and groups it with a diverse brain disorders such as prion encephalopathies, Alzheimer's disease, and Parkinson's disease, although the aggregation sites differ [38]. Direct evidence for misfolded mutant Htt lies in the fact that certain antibodies are able to distinguish between the mutant and normal forms of the protein [28].

A misfolding of the mutant Htt protein due to the extended N-terminal could alter protein function, as the polyQ stretch may

Table 2. Huntingtin Interacting Proteins [39,143-145].

Name	Protein function	Htt binding region	Influence of mu HTT
Transcription			
CA150	Transcription activator	Unknown	None
CBP	Transcription activator	Amino acids 1-588	Enhances
CtBP	Transcription repressor	Unknown	Decreases
HYP-A, B	RNA splicing factors	Polyproline	Enhances
HYP-C	Transcription factor	Polyproline	Enhances
NCOR	Transcription repressor	Amino acids 1-171	Enhances
NF-κB	Transcription factor	HEAT repeats	Unknown
SP1	Transcription activator	Amino acids 1-171	Enhances
TAFII130	Transcription activator	Amino acids 1-480	None
TBP	Basal transcription factor	Unknown	Unknown
P53	Transcription factor	Polyproline	None
REST-NRSE	Trascription suppressor	Amino acids 1-548	Decreases
Trafficking and endocytosis			
HAP1	Trafficking, endocytosis	Amino acids 1-230	Enhances
HIP1	Endocytosis, pro-apoptotic	Amino acids 1-540	Decreases
HIP14	Trafficking, endocytosis	Amino acids 1-550	Decreases
PACSIN1	Endocytosis	Polyproline	Enhances
Phosphatidylethanolamine PI(3,4,5)P3	Phospholipids	Amino acids 171-287	Enhances
PSD-905	Synaptic scaffolding	Unknown	Decreases
Signaling			
Calmodulin	Calcium-binding regulatory protein	Unknown	Enhances
CIP-4	Cdc42-related signaling	Amino acids 1-152	Enhances
FIP2 (HYP-L)	GTPase Rab8 interactor	Amino acids 1-550	Unknown
GRb2	Growth factor signaling	Polyproline	Unknown
IP31	Calcium release channel	Amino acids 1-158	Enhances
SH3GL3	Endocytosis and vesicle recycling	Polyproline	Enhances
RasGAP	Ras GTPase-activating protein	Polyproline	Unknown
Metabolism			
Cystathionine b-synthase	Generation of cysteine	Amino acids 1-171	None
GAPDH	Glycolitic enzyme	Polyproline	Enhances
gp78	ER membrane-anchored ubiquitin ligase	HEAT repeats 2/3	Enhances
HIP2	Ubiquitin-conjugated enzyme	Amino acids 1-540	None
Protein Synthesis			
Gnb211	Translation (indirect,) ribosomal protein	Unknown	Decreases
Myo5a	RNA transport to spines	Unknown	Decreases
Rps6	Translation (direct), Ribosomal protein	Unknown	Decreases
Prkra	Translation (indirect), PKR regulation	Unknown	Decreases

Abbreviations: CA150, co-activator 150; CBP, (cAMP-response element binding protein) binding protein; CIP-4, cdc42-interacting protein 4; Co-IP, co-immunoprecipitation; CtBP, C-terminal-binding protein; FIP2, for 14.7K interacting protein; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; Gnb2l1, guanine nucleotide-binding protein (G protein) polypeptide 2-like 1; gp78, glycoprotein 78; GRB2, growth factor receptor-binding protein 2; GST, glutathione S-transferase; HAP1, htt-associated protein 1; HIP, htt-interacting protein; HYP, htt-yeast partner; IP31, inositol (1,4,5)-trisphosphate receptor type 1; Myo5a, myosin V;A NCOR, nuclear receptor co-repressor; NF-kB, nuclear factor-kB transcription factor; PACSIN1, protein kinase C and casein kinase substrate in neurons 1; [PI(3,4,5)P3] phosphoinositol, (PI) 3,4-bisphosphate, PI 3,5-bisphosphate, and PI 3,4,5-triphosphate; PKR, double-stranded RNA-activated protein kinase; Prkra, interferon inducible double-stranded RNA-dependent protein kinase activator A; PSD- 95, postsynaptic density 95; RasGAP, Ras GTPase-activating protein; REST–NRSE, the repressor element-1 transcription factor–the neuron restrictive silencer element; Rps6, ribosomal protein S6; SH3GL3, SH3-containing GRB2-like protein 3; SP1, specificity protein-1; TAFII130, TBP-associated factor; TBP, TATA box binding protein.

present itself or nearby Htt domains in a more or less provocative way to potential binding partners. Indeed, an assessment of the changes in mutant Htt's protein interactions shows that HD has elements of loss-of-function occurring at the same time as gain-of-function, both perturbing normal Htt functions and gaining deleterious new cellular activities [39]. These altered binding partner relationships are all potential therapeutic targets.

Being a large protein, Htt has numerous binding partners, including transcription co-activators [40], co-repressors [41], and apoptosis-related kinases [42]. Htt's normal function may impact many cellular processes, including signal transduction, endocytosis, cytoskeletal structure, transcription and axonal transport [43-45], and in the presence of the expanded polyQ stretch, the interactions with binding partners can be increased or decreased (Table 2). Interestingly, expression of the N-terminal section of mutant Htt is enough to cause neuronal degeneration, but is not sufficient to maintain Htt's axonal transport functions [46].

Htt was the first neurodegenerative disease protein to be identified as a caspase substrate [47]. Htt can be cleaved by caspases (including caspase-2 [48] and caspase-3 [47]), calpain [49], and the matrix metalloproteinase MMP-10 [50] at a regional "hot spot" within Htt between 400 and 600 amino acids, resulting in N-terminal Htt fragments that are small enough to passively translocate into the nucleus. Once inside the nucleus, the mutant Htt cleavage product can form nuclear inclusions that recruit transcription factors, and soluble mutant Htt can aberrantly repress transcription itself [18].

Proteolytic processing is likely an important initial step in pathogenesis, since expressing the smaller Htt truncation product results in greater cell toxicity than expressing the entire mutant htt protein [51,52] and inhibition of cleavage can lessen neurotoxicity in animal models [53]. Htt contains a strictly conserved nuclear export signal that is cleaved away in HD [54], and nuclear targeting of mutant Htt increases toxicity [55],

so devising a way to keep mutant Htt out of the nucleus could be beneficially therapeutic.

In addition to cleavage, Htt is normally subject to several types of post-translational modifications, including acetylation, phosphorylation, methylation, sumoylation, and ubiquitination, which can be altered in the presence of the expanded allele [56]. For example, Htt phosphorylation at serine 421 promotes anterograde transport within the neuron, but this function is impaired in the presence of the mutant allele [57]. Htt phosphorylation at serines 13 and 16 can protect against expanded polyQ toxicity [58], and increasing or mimicking phosphorylation at these sites is currently an area of therapeutic investigation.

BIOCHEMICAL MODIFIERS

Although the certainty of a genetic diagnosis can be daunting for affected patients and their families, early genetic testing of individuals at risk for an autosomal dominant disorder would allow ample time for a potential therapy to be administered [59]. However, with no available treatment, and with more than 10 potential disease-modifying drugs showing no significant difference in clinical trials [58], many at-risk individuals choose not to find out their genetic status.

In 2000, the first regulatable mouse model of HD showed that it is possible to reverse aggregate formation and disease symptoms after they have manifested [60], which gave researchers hope that a potential therapy could even be effective in symptomatic patients. The cumulative damage hypothesis states that in neurodegenerative disease, neurons are slowly overwhelmed by accumulated damage (such as that caused by oxidative stress or toxic protein accumulation), and those neurons become increasingly committed to an apoptotic future. On the contrary, the "one-hit" biochemical model of several inherited diseases, including HD, uses statistical analysis to argue against the cumulative damage hypothesis [61]. The "one-hit" model proposes that a

single catastrophic intracellular event results in neuronal death. At any one time, each neuron is at constant risk of cell death, which implies that treatment administered at any disease stage should be beneficial.

In animal models, we can delay the disease process and even reverse it — we can dismantle aggregates and rescue phenotypes [60,62-65]. Theoretically, if we can do it in animals, in humans we should be able to administer a small molecule drug to keep pre-symptomatic patients healthy or to restore proper neuronal function at any stage of the disease, as long as the neurons are still present. However, the post-mitotic nature of neurons, combined with difficulties crossing the blood brain barrier, pose substantial hurdles to effectively reaching the striatal target cells. Recent advances in transforming adipose-derived stem cells derived from HD patient into pluripotent stem cells (that can be transformed into neurons) should assist with elucidating disease mechanisms and hasten the testing of small molecule therapies [66].

One such small molecule under investigation aims to inhibit histone deacetylases (HDACs). Truncated mutant Htt can inhibit, mislocalize, and degrade acetyltransferases, which are enzymes that normally modify proteins to increase gene activity [67]. This interaction is mediated through the proline rich domain, as well as the polyQ stretch, and results in reduced levels of acetylated histones [68]. HDACs are able to reverse this reduction and reduce lethality in animal models of HD, even after symptom onset, so HDAC inhibitors are another potential treatment, as they can influence not only gene transcription but also potentially alleviate endoplasmic reticulum stress or modulate chaperone activity [67].

The HD phenotype could conceivably be delayed by preventing aggregate formation and/or increasing aggregate clearance by targeting proteosome function, increasing ubiquitination, or increasing autophagy. Eukaryotic cells have two pathways for clearance — under normal circumstances, the ubiquitin/proteosome system functions at high levels, whereas the autophagy/lyso-some system maintains low activity levels [69]. If mutant Htt overwhelms the ubiquitin/proteosome system in HD, the neuron will then induce autophagy for protein clearance [70].

Indeed, the autophagic response is one of the first neuronal responses to mutant Htt [71] and is predominately responsible for clearing the cytoplasmic aggregates [72]. Polymorphisms in autophagy-related genes contribute to the age of onset in HD [73]. Remarkably, Htt may normally regulate mechanisms of protein degradation that are ultimately involved in its own clearance [74], and in the disease process, expression of Htt with a deleted polyQ tract in a 140Q/+ knock-in mouse model can upregulate autophagic markers and increase lifespan [65]. Unlike the cytoplasmic aggregates, HD nuclear inclusions appear to be cleared using the ubiquitin/proteosome system instead [69], making both the autophagic and the ubiquitin/proteosome systems attractive therapeutic targets.

MODULATING EXCITOTOXICITY AND METABOLISM

Excitotoxicity has been implicated in the selective neuronal death seen in HD [75]. There is an intimate relationship between cellular metabolism and excitotoxicity. Although implicated in HD several ways, the strongest evidence that mitochondria are involved in HD pathogenesis is the fact that administration of the mitochondrial toxin 3-nitropropionic acid (3-NP) can mimic HD characteristics [76], including selective cell death in the striatum, cognitive impairment, and the development of motor symptoms in a non-human primate model [77].

Both creatine and coenzyme Q10 administration reduce reactive oxygen species to address the metabolic defects in HD. Creatine is involved with energy buffering and the connection between energy production and consumption within the cell. When orally administered in a HD mouse model, creatine improves survival and delays atrophy and aggregate formation [78]. Coen-

zyme Q10 is an antioxidant, as well as an essential part of the electron transport chain. Coenzyme Q10 can alleviate symptoms, extend survival time, and slow striatal atrophy in a mouse model of HD [76]. Unfortunately, limited efficacy of these agents has been observed in HD patients; however, the optimal therapeutic dose may have been underestimated and higher dose administration is under investigation [73]. Another metabolic therapeutic target is the P2X receptor, part of the signaling machinery mediating ATP responses to neurodegenerative stressors [79].

Growth factors and cytokines play a role in HD pathology and may particularly modulate the effects of excitotoxicity. Transforming growth factor β1 (TGF-β1) is reduced in cortical neurons of HD patients and mouse models [80], so supplementation is a possibility, or perhaps TGF-β1 could be a useful biomarker for disease progression.

Retroviral administration of ciliary neurotrophic factor (CNTF) can alter the neuronal degeneration and prevent deficits in an excitotoxic HD rat model [81,82]. Bilateral striatal implantations of CNTF releasing cells into a primate model of HD at symptom onset protects neurons from further degeneration, as well as offering cognitive and motor improvement [83]. Peripheral administration of CNTF is not well tolerated due to side effects, but implantation of CNTF-releasing cells remains a possibility [84]. A phase I study implanting a device with a semi-permeable membrane encapsulating a cell line engineered to synthesize CNTF in HD patients showed that administration within the ventricle is safe and feasible; however, the technique needs improvement, as CNTF levels were low in many patients and there were varying cell survival numbers within capsules after removal [85].

Brain-derived growth factor (BDNF) is a neurotrophic factor speculated to play a role in neuronal development and survival [86], and BDNF can prevent cell death in excitotoxic models of HD [87]. One of normal Htt's regular jobs in the cell is to bind up transcriptional repressors (such as REST–NSFR [the repressor element-1 transcription factor–the neuron restrictive silencer element]) in the cytoplasm. Mutant Htt does a poor job of binding to the repressors, resulting inhibition of target genes, including brain-derived neurotrophic factor (BDNF) [73]. Not surprisingly, a 53 percent to 82 percent reduction in BDNF expression was found in the striatum of HD patients upon autopsy [88]. Enrollment was recently completed for a phase 2/3 clinical trial investigating cysteamine bitartrate delayed-release capsules (RP103) for HD in France, following results showing that cysteamine increases BDNF levels in rodents and primate models of HD [89].

Glial cells play a major role in local trophin availability, and astrocytes are able to both respond to and produce BDNF [90]. Efforts can be made to increase gene products like BDNF, for example, astrocytes engineered to overexpress BDNF are being explored as a potential gene therapy in a rodent model of HD [91].

A ROLE FOR ASTROCYTES IN HD

Recently, the research focus on HD neuronal dysfunction has expanded to include a possible glial role in pathogenesis. Glial cells do express Htt [17] and original pathology work showed marked gliosis as a disease marker, becoming more widespread as the disease progresses [92]. Mouse models expressing mutant Htt show glial nuclear aggregates [93], and specific astrocytic expression of 160Q N-terminal mutant Htt fragments can induce neurological symptoms in mice [94]. Interestingly, mouse stem cells expressing no Htt are much more likely to differentiate into glial cells than cells expressing Htt with 20, 50, 111 or 140 polyQ repeats, even when treated with the same *in vitro* neural differentiation protocol [95].

Neurons are dependent on astrocytes metabolically and cooperate very closely with astrocytes when it comes to circumventing glutamate-mediated excitotoxicity [96], so investigating this relationship in HD seems reasonable. Nearly 80 percent of glutamate is removed from the synapse by the astrocytic transporters glutamate transporter

1 (GLT1) and glutamate-aspartate trans-porter (GLAST) [97]. Interestingly, knock-outs of either of these glial glutamate transporters results in excess extracellular glutamate levels and a progressive motor phenotype [98]. If the GLT1 transporters are nonfunctional or missing, neuronal damage due to glutamate over-stimulation is likely to occur [99,100], and indeed, decreased mRNA levels for GLT1 are found in HD [101].

Astrocytes are also responsible for sup-plying adult neurons with cholesterol [102], a key ingredient for normal synaptogenesis and neurotransmitter release [103,104]. Cholesterol biosynthesis is reduced in astro-cytes isolated from HD mouse models [105], though Htt's involvement in cholesterol homeostasis remains to be fully elucidated. Since astrocytes are very sensitive to cues in the environment surrounding the neurons, they may also be affected by increased cili-ogenesis caused by mutant Htt [106]. The non-motile cilia have a sensory role in reg-ulating signaling pathways, such as hedge-hog and PDGF-α [106]. The restoration of normal ciliary function — though certainly not a complete treatment — could be a po-tential therapeutic target.

GENETIC MODIFIERS

Early investigations showed that a gene closely linked to the *HD* gene may modify age of onset [107]. Targeting cis-regulatory elements to delay the appearance of symp-toms is a strategy that remains to be eluci-dated, as the exact nature of these regulatory elements are still unknown. However, se-quence variations in the PPARGC1A gene encoding PGC-1α (involved in mitochondr-ial function), as well as polymorphisms in PGC1α's downstream targets, can exert modifying effects on the age of onset in HD [108]. Subtypes of N-methyl D-aspartate re-ceptor genes (GRIN2A and GRIN2B) may also modify age of onset [109].

The length of Htt's polyQ stretch in the normal allele does not influence when HD symptoms first appear [110], which suggests that strategies to decrease Htt expression it-self may be effective. This proposal is more complicated than simply ridding the cell of a benign protein that has turned noxious. The development of knockout and condi-tional knockout mouse models demonstrate that Htt is essential for early embryogenesis [20-22] and spermatogenesis [111]. Rather than seeing a decrease in Htt expression fol-lowing execution of its critical role in em-bryogenesis, postnatal Htt expression levels actually rise in the adult [112]. Htt plays a critical role in the development of proper neuronal connections and apoptosis [113], and it is not yet known if lack of Htt expres-sion as an adult would be benign.

Existing literature suggests that Htt loss of function may comprise essential neu-rodevelopment programs, including neu-ronal organization through a pivotal role in mitotic spindle orientation [114] and neu-ronal maturation via its role in ciliogenesis [115]. Htt itself has antiapoptotic properties [116], and depletion of wild type Htt has been found not just in mouse models of HD, but also in models of neurodegeneration sec-ondary to ischemia and traumatic brain in-jury [117]. Eliminating expression without a complete understanding of Htt's normal cellular function could confound the plight of already sick neurons.

The *HD* gene can be silenced *in vivo* using RNA strategies (small interfering RNA [siRNA] or short hairpin RNA [shRNA]) or by antisense oligonucleotides (ASO). Temporally sensitive administration of RNA therapy could reduce Htt production in HD gene carriers and potentially elimi-nate Htt protein in adult tissues. However, practical applications of these therapies struggle to find the best routes of adminis-tration, due to the blood brain barrier, and the appropriate cells to target, as we know that HD is not solely a striatal specific dis-ease [118]. In addition, gene suppression strategies must be carefully designed to avoid off-target effects and dosage control.

Small-scale siRNA knockdown experi-ments in monkeys [119] and mice using siRNA [120] appear promising; however, in the process of eliminating Htt protein ex-pression, wild-type Htt protein expression is

often decreased as well. Continuous partial suppression of both forms of Htt expression in rodent models decreases neuropathology, reduces symptoms, and prolongs survival, even when wild-type Htt was also eliminated [121,122]. To selectively target just the mutant Htt allele, RNA strategies can capitalize on the presence of the expanded polyQ [123] or on the presence of single nucleotide polymorphisms (SNPs) associated with the presence of the expanded polyQ allele [124] as targets. The recent achievement of an allele-selective siRNA in an HD mouse model may make the siRNA technique the most effective way forward for Htt silencing efforts [125].

But can these knockdown techniques be translated effectively from mice to humans? Recently, a convection-enhanced delivery system delivered 7 days of a siRNA treatment to the much larger non-human primate brain and was able to decrease Htt expression effectively throughout the striatum, removing a technical delivery hurdle for human therapy translation [126]. Still more promising, favorable results from a phase 1 trial that used siRNA to block the expression of SOD1 in familial forms of Amyotrophic Lateral Sclerosis (ALS) were presented at the 2012 American Academy of Neurology Annual Meeting. In humans affected with HD, neurons are able to sustain the expression of mutant Htt for many years before aggregates form and neurodegeneration begins. It may be that a brief elimination of mutant Htt synthesis is all a neuron needs to get a better handle on clearing the mutant Htt from the cell and to keep symptoms at bay.

HUNTINGTIN AS A DEVELOPMENTAL DISORDER

Although HD patients are usually not symptomatic until mid-life, abnormal brain development may contribute to HD pathophysiology. Affected patients often experience weight loss that pre-dates motor abnormalities [127], and brain scans reveal enlarged cortex size [128] and decreased intracranial volume [127] in presymptomatic patients, which suggests abnormalities in

neural development. Presymptomatic children carrying the HD expansion have lower body mass index (BMI) and head circumference than controls, suggesting defects with energy regulation and brain growth are present 30+ years before overt symptoms would normally appear [129].

These findings group HD into yet another disease category (with disorders such as schizophrenia, familial Alzheimer's disease, and SCA-1), where abnormal development sets the stage for a later stressor, resulting in cell death [130]. The evidence for very early disease manifestation, in combination with Htt's essential roles in early development, brings HD full circle from a neurodegenerative disease to a developmental disease with cellular homeostasis defects that predispose neurons to die in mid-life [131].

Htt, as a fairly social protein within the cell, may have multiple roles to play during development and during adulthood, and different protein domains may be essential at distinct time points [132]. Even normal variations in the size of the Htt polyQ stretch are associated with differences in brain measurements in the pallidum, with longer repeat lengths associated with more grey matter in normal individuals [133]. More research is needed to discover the normal functions of Htt at multiple stages in development. Thinking about HD as a type of developmental disorder opens the door for very early intervention targets, and yet pointedly introduces the issue of responsible genetic testing of juveniles with no available cure.

CONCLUSION

It has been more than a century since the first description of the disease was published. With the advent of molecular genetics techniques, we now are able to view a blueprint of each individual's DNA. In a few short decades, HD diagnosis became as easy as a blood test. But Htt turns out to have multiple roles within the cell, and the HD story has been far more complex than most imagined. Many of the original pioneering HD researchers are still working on this dis-

ease, and the hope is that a physician will be able to hand patients an effective treatment along with an HD diagnosis. The HD gene was the first to be mapped; perhaps it will also be the first neurodegenerative disease to be cured.

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