Huntington’s disease: from molecular pathogenesis to clinical treatment

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Huntington’s disease is a progressive, fatal, neurodegenerative disorder caused by an expanded CAG repeat in the huntingtin gene, which encodes an abnormally long polyglutamine repeat in the huntingtin protein. Huntington’s disease has served as a model for the study of other more common neurodegenerative disorders, such as Alzheimer’s disease and Parkinson’s disease. These disorders all share features including: delayed onset; selective neuronal vulnerability, despite widespread expression of disease-related proteins during the whole lifetime; abnormal protein processing and aggregation; and cellular toxic effects involving both cell autonomous and cell-cell interaction mechanisms. Pathogenic pathways of Huntington’s disease are beginning to be unravelled, offering targets for treatments. Additionally, predictive genetic testing and findings of neuroimaging studies show that, as in some other neurodegenerative disorders, neurodegeneration in affected individuals begins many years before onset of diagnosable signs and symptoms of Huntington’s disease, and it is accompanied by subtle cognitive, motor, and psychiatric changes (so-called prodromal disease). Thus, Huntington’s disease is also emerging as a model for strategies to develop therapeutic interventions, not only to slow progression of manifest disease but also to delay, or ideally prevent, its onset.

Introduction

Huntington’s disease can be regarded as a model neurodegenerative disorder. It is monogenic, fully penetrant, and—similar to other neurodegenerative diseases—a disorder of protein misfolding. The gene for Huntington’s disease, huntingtin (HTT), was discovered 17 years ago, and much has been learned about the disease’s pathogenesis since then. Huntington’s disease is caused by a CAG triplet repeat expansion in HTT, which encodes an expanded polyglutamine stretch in the huntingtin (HTT) protein. The disease is inherited in an autosomal dominant manner with age-dependent penetrance, and repeat CAG lengths of 40 or more are associated with nearly full penetrance by age 65 years. Individuals at risk of inheriting the expanded CAG nucleotide can be identified before clinical onset by predictive genetic testing. Longer CAG repeats predict earlier onset, accounting for up to 50–70% of variance in age of onset, with the remainder likely to be due to modifying genes and the environment. By contrast, length of the CAG repeat seems to contribute less to the rate of progression, and understanding the determinants of rate of progression could provide means for intervention.

Prevalence of Huntington’s disease is 4–10 per 100 000 in the western world, with many more people at risk of the disease. Mean age of onset is 40 years, with death occurring 15–20 years from onset (figure 1). Clinical features of Huntington’s disease include progressive motor dysfunction, cognitive decline, and psychiatric disturbance, probably caused by both neuronal dysfunction and neuronal cell death. Formal diagnosis of Huntington’s disease is made on the basis of characteristic extrapyramidal motor signs of chorea, dystonia, bradykinnesia, or incoordination in an individual at risk. Although chorea is usually prominent early in the course of the disease, later progressive bradykinnesia, incoordination, and rigidity (so-called motor impairment) are more disabling functionally. Many patients have substantial cognitive or behavioural disturbances before onset of diagnostic motor signs. Most drugs currently used for symptomatic management of Huntington’s disease (table) are derived from anecdotal clinical experience. In a randomised controlled trial, tetrabenazine reduced chorea. Behavioural and social interventions are often as effective as drug treatments for behavioural difficulties.

Identification of new targets, strategies for drug discovery, and therapeutic approaches are now reaching an important turning point. Methods leading to successful development and testing of rational neuroprotective (disease-modifying) treatments are on the horizon. Furthermore, identification of biomarkers in individuals positive for the Huntington’s disease expansion mutation, who may have subtle cognitive motor or emotional signs and symptoms, but prior to sufficient motor signs for a formal diagnosis (prodromal disease), suggests that preventive treatment could be possible.

Our Review covers the pathogenesis of Huntington’s disease relevant to current and potential future therapeutic targets and the translation of this work to clinical trials. We highlight relevant areas of progress and principles, questions, and challenges ahead in trying to develop and test such treatments in patients, particularly before functional impairment happens, when neuronal dysfunction and other neurobiological abnormalities are most likely to be still reversible.

Principles of pathogenesis

HTT is a very large protein predicted to consist mainly of repeated units of about 50 amino acids, termed HEAT repeats (figure 2). These repeats are composed of two antiparallel α-helices with a helical hairpin configuration, which assemble into a superhelical structure with a continuous hydrophobic core. HTT has many interaction
partners, particularly at its N-terminus,\textsuperscript{7} suggesting that it serves as a scaffold to coordinate complexes of other proteins. HTT also undergoes extensive post-translational modification (figure 2).

The cellular functions of HTT are still not completely understood.\textsuperscript{1,3,19} The protein is mostly cytoplasmic, with membrane attachment via palmitoylation at cysteine 214.\textsuperscript{40} A putative nuclear export signal is present near the C-terminus but a clear nuclear localisation signal has not been identified. HTT shuttles into the nucleus, has a role in vesicle transport, and can regulate gene transcription.\textsuperscript{19,21} It might also regulate RNA trafficking.\textsuperscript{3}

Most available evidence—including dominant genetic transmission, presence of abnormal aggregated proteins, and findings of biochemical, cell, and mouse model studies—suggests that Huntington’s disease arises predominantly from gain of a toxic function from an abnormal conformation of mutant HTT.\textsuperscript{3,4} The RNA might also have toxic properties, and loss of function of HTT could also contribute to disease pathogenesis,\textsuperscript{15} perhaps entailing antisense RNA. Furthermore, HTT is necessary for early embryonic development. Mutant HTT (eg, via transgenic expression) can complement loss of function (eg, via knockout) of HTT during development, consistent with the idea that the Huntington’s disease phenotype does not arise predominantly from loss of HTT function. Findings of recent studies have suggested that the presence of the mutant protein in a knock-in mouse model with 111 CAG repeats (Q111) leads to transient early developmental abnormalities, which the researchers suggest compromise neuronal homeostasis and subsequently render medium spiny neurons more vulnerable to late life stressors.\textsuperscript{25}

Key features of Huntington’s disease pathogenesis have been described consistently (see also Selected mechanisms, targets, and experimental treatments). First, mutant HTT has the propensity to form abnormal conformations, including β-sheet structures (although HTT in large inclusions is not the primary pathogenic species in Huntington’s disease). Second, systems for handling abnormal proteins are impaired in cells and tissues from Huntington’s disease patients or models. Third, HTT is truncated and gives rise to toxic N-terminal fragments. Fourth, post-translational modifications of HTT influence toxicity, via conformational changes, aggregation propensity, cellular localisation, and clearance. Fifth, nuclear translocation of mutant HTT enhances toxic effects of the protein, in part via transcription-related effects. Finally, cellular metabolic pathways are impaired in samples from Huntington’s disease patients and models (see Metabolism; figure 3).

Some of these pathways could offer especially good therapeutic targets for drug development. They will be discussed in detail below (see Selected mechanisms, targets, and experimental treatments).

Much of what we know about Huntington’s disease biology arises from study of model systems, ranging from those in cells and invertebrates to mammals (panel 1). We should keep in mind not only the strengths of these models but also their limitations. The ultimate test of disease models will be the extent to which biomarkers and therapeutic effects correspond among model systems and human disease.

Most clinical features of Huntington’s disease can be attributed to CNS degeneration, but some aspects of the disease could be mediated outside the CNS,\textsuperscript{31–33} including weight loss and muscle wasting, metabolic dysfunction, and endocrine disturbances. Within the brain, there is massive striatal neuronal cell death,\textsuperscript{34,35} with up to 95% loss of GABAergic medium spiny projection neurons, which project to the globus pallidus and the substantia nigra, whereas large interneurons are selectively spared. Furthermore, there is atrophy of the cerebral cortex, subcortical white matter, thalamus, specific hypothalamic nuclei, and other brain regions, though less severe than in the striatum. In advanced cases, especially with juvenile onset, there is widespread brain atrophy. The pathognomonic pathological signature of Huntington’s disease consists of intranuclear inclusion bodies, which are large aggregates of abnormal HTT in neuronal nuclei (figures 3 and 4). Aggregates also arise
elsewhere in the cell, including the cytoplasm, dendrites, and axon terminals.\textsuperscript{35,36} Density of visible aggregates does not correlate well with distribution of cell death, consistent with the idea that they are, in part, a protective cellular response to misfolded protein (see Conformation and aggregation of HTT).

Why does relative striatal selectivity take place, despite widespread expression of HTT throughout the brain and body? Hypotheses include susceptibility to loss of brain-derived neurotrophic factor (BDNF) neurotrophic support from cortical-striatal projections or, conversely, susceptibility to excitotoxicity also arising from cortical glutamatergic projections.\textsuperscript{37,38} A recent proposal for a possible interaction between HTT and the Rhes protein (see Post-translational modifications of HTT)\textsuperscript{39} might account for striatal selectivity. Rhes is expressed preferentially in the striatum and is expressed at diminished levels in other forebrain areas that are affected in Huntington’s disease. However, its relative expression in medium spiny neurons versus other neurons has not yet been clearly defined, and it is expressed in regions that are not known to be affected in Huntington’s disease, such as the superior colliculus and granule cells of the cerebellum. Most data on the interaction of HTT and Rhes to date come from in-vitro studies, so further in-vivo work will be necessary.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Class</th>
<th>Main adverse effects and treatment notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chorea</td>
<td>Tetrabenzine</td>
<td>Depression and sedation</td>
</tr>
<tr>
<td>Myoclonus, chorea, dystonia, rigidity, spasticity</td>
<td>Clonazepam, Levetiracetam</td>
<td>Sedation, ataxia, apathy, cognitive impairment could be exacerbated, withdrawal seizures</td>
</tr>
<tr>
<td>Myoclonus</td>
<td>Sodium valproate, Levetiracetam</td>
<td>Gastrointestinal disturbance, weight gain, blood dyscrasia, hyperammonaemia, liver dysfunction</td>
</tr>
<tr>
<td>Rigidly (particularly associated with juvenile Huntington’s disease or young adult-onset parkinsonian phenotype)</td>
<td>Levodopa</td>
<td>Gastrointestinal disturbance, postural hypotension, insomnia, agitation, psychiatric symptoms, increased chorea</td>
</tr>
<tr>
<td>Rigidly, spasticity</td>
<td>Baclofen, tizanidine</td>
<td>Sedation, drowsiness, confusion, gastrointestinal disturbances, hypotension</td>
</tr>
<tr>
<td>Bruxism, dystonia</td>
<td>Botulinum toxin</td>
<td>Inhibits acetylcholine release at neuromuscular junction to cause muscle paralysis</td>
</tr>
<tr>
<td>Psychosis, irritability</td>
<td>Olanzapine, Quetiapine</td>
<td>Sedation, parkinsonism, tardive dyskinesia, and neuropsychiatric symptoms, but less risk of these than with older neuroleptics, raised triglycerides, weight gain from increased appetite, which could be beneficial (in relation to the weight loss seen in Huntington’s disease). Caution should be exercised in patients with diabetes, and blood glucose should be monitored. Might rarely cause prolonged QT interval. Useful if patient also has agitation, irritability, and anxiety</td>
</tr>
<tr>
<td>Psychosis, chorea, irritability</td>
<td>Risperdone, Sulpiride, Haloperidol</td>
<td>As above for olanzapine, but less effect on increasing appetite</td>
</tr>
<tr>
<td>Treatment-resistant psychosis</td>
<td>Clozapine</td>
<td>As for other neuroleptics, plus agranulocytosis, myocarditis, and cardiomyopathy. Needs blood monitoring</td>
</tr>
<tr>
<td>Psychosis with prominent negative symptoms</td>
<td>Aripiprazole</td>
<td>Parkinsonism, akathisia, drowsiness, gastrointestinal disturbance, tremor, blurred vision</td>
</tr>
<tr>
<td>Depression, anxiety, obsessive compulsive behaviour, irritability</td>
<td>Citalopram, Fluoxetine, Paroxetine, Sertraline, Mirtazapine, Venlafaxine</td>
<td>Gastrointestinal disturbance, hypersensitivity reactions, drowsiness, syndrome of inappropriate antidiuresis, postural hypotension</td>
</tr>
<tr>
<td>Altered sleep-wake cycle</td>
<td>Zopiclone, zolpidem</td>
<td>Hypnotics, Drowsiness, confusion, memory disturbance, gastrointestinal disturbance</td>
</tr>
<tr>
<td>Mania or hypomania</td>
<td>Sodium valproate, Carbamazepine</td>
<td>Hypersensitivity reactions, drowsiness, blood dyscrasia, hepatitis, hyponatraemia, dizziness, gastrointestinal disturbance</td>
</tr>
<tr>
<td>Lithium</td>
<td>Mood stabiliser</td>
<td>Renal insufficiency, hypothyroidism, and tremor, with a narrow therapeutic window, and overdose can cause delirium and renal failure</td>
</tr>
</tbody>
</table>

Table: Symptomatic drug treatment for Huntington’s disease

SSRI=selective serotonin reuptake inhibitor. Adapted from ref 10, with permission of BMJ Publishing Group.
The length of the CAG repeat accounts for only about 50–70% of the overall variance in age of onset, and less for later onset cases. Only a few linkage and association studies have been done to date, in which several candidate modifier genes were identified, including HAP1, GRIK2 (formerly GLUR6), and TCERG1 (formerly CA150). Further systematic studies of larger samples for linkage or genome-wide association studies, or resequencing of families, could potentially yield additional therapeutic targets. Importantly, several genetic modifiers code for proteins known to interact with HTT (eg, HAP1 [huntingtin-associated protein 1] and CA150 [transcription elongation regulator 1]) or are believed to be in Huntington’s disease pathogenic pathways (eg, GLUR6 [glutamate receptor ionotropic, kainate 2] and PGC1α [peroxisome elongation regulator 1]).

Selected mechanisms, targets, and experimental treatments

The directionality and sequence of pathogenic events in Huntington’s disease is still poorly understood. Ideally, therapeutic interventions would target early steps in a pathogenic chain of events. With our currently limited knowledge, it is difficult to identify the crucial steps (after those that include HTT) in the pathogenic pathways. Furthermore, some cellular effects, which might appear relatively far downstream, such as alterations in cellular metabolism (figure 3), could feed back to influence early steps in the pathogenic pathway. Cells with impaired energy supplies due to proteotoxic stress might be unable to handle toxic forms of mutant HTT so metabolic therapies could affect early stages of pathogenesis. We should be open-minded about what kind of screening approaches will be most likely to generate therapeutic leads (panel 2). A compendium of mechanisms and targets can be found on the Huntington’s disease Research Crossroads website, which summarises target validation data for more than 600 genes and includes data for compounds and interventions.

Transcription, translation, and clearance of mutant HTT

One therapeutic strategy in gain-of-function neurodegenerative diseases is to reduce the amount of pathogenic protein—either by decreasing production or by increasing clearance. Shutting off expression of mutant HTT in an inducible transgenic mouse system led to partial recovery of both behavioural and pathological features, although little further study of this model has been done. Since the HTT gene seems to have a so-called housekeeping promoter (ie, a promoter yielding widespread constitutive expression with little regulation), own, or exacerbates the neuronal phenotype in the N171-82Q model (panel 1). Possibilities for exploitation of cell interactions for therapeutic development include inflammation or excitotoxicity (see Excitotoxicity, inflammation, and the quinolinic acid pathway). The striatum receives massive neuronal projections from the cortex, releasing glutamate as the neurotransmitter and BDNF as an important neuromodulator and trophic factor (figure 4). These pathways could contribute to selective striatal vulnerability and might be valuable therapeutic targets.

Currently, we do not understand fully the extent to which signs and symptoms of Huntington’s disease arise from cell death compared with cell dysfunction. The early predominance of chorea has been ascribed to differences in cell death in striatal output subcircuits (eg, timing of degeneration of neurons containing enkephalin vs substance P). However, another possibility might be that neuronal dysfunction happens before cell death (figure 1).

This distinction would have many implications for timing of different therapeutic strategies.

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**Figure 2: HTT domain structure and post-translational modifications**

Human HTT is predominantly composed of HEAT repeats. A polyglutamine stretch (polyQ) is located at the N terminus. Proteolytic cleavage—by caspase 6 and other (as yet, uncharacterised) proteases—forms toxic fragments, examples of which are shown (eg, cp-1 and cp-2). The exact size of these fragments and the relevant cleavage enzymes are currently unknown. Many post-translational modifications (eg, acetylation [Ac], phosphorylation [P], and addition of small ubiquitin-like modifiers [SUMO]) can alter HTT’s cell biology and toxic effects. IVLD and NLPR are amino acid cleavage sequences. NES=nuclear export signal.
targeting for selective downregulation of HTT transcription could be difficult. However, HTT mRNA might be a productive target.

Reduction of amounts of mutant HTT in the brain can be achieved via targeted small interfering RNA (siRNA) or antisense oligonucleotides. Use of siRNA can decrease mutant HTT expression and ameliorate the phenotype in mouse models of Huntington’s disease, and promising results have been shown with antisense oligonucleotides infused directly into the lateral ventricles of mouse models of Huntington’s disease. Many different strategies are being tested to establish optimum delivery methods for antisense and siRNA treatments.

We do not know the degree of reduction in wild-type HTT in the adult brain that can be tolerated in the long term. Conditional deletion of HTT in the forebrain of adult mice lead to neuronal degeneration, therefore, therapeutic attempts to reduce mutant HTT must be careful not to lower the amount of wild-type HTT excessively. Selective reduction in amounts of mutant HTT mRNA might be possible without affecting the normal allele. Preclinical validation studies targeting mutant HTT expression are underway and represent an exciting therapeutic possibility. However, for chronic treatment of Huntington’s disease, long-lasting interventions would be necessary, with either continuous or repeated long-term intraventricular infusion of agents, or difficulties associated with viral delivery and safe stable expression.

Half-life and clearance of normal and mutant HTT have not been studied in detail. Cells have compensatory mechanisms against unfolded and abnormal proteins (figure 5), and enhancement of these responses might be possible. Two major cellular pathways for degradation of misfolded proteins are the ubiquitin-proteasome system and autophagy. Researchers have postulated that a toxic effect of mutant HTT could be to compromise ubiquitin-proteasome activity. Changes in the ubiquitin system in Huntington’s disease mouse model and human post-mortem brain tissue might represent cellular anomalies or an appropriate cellular response to the abnormal protein. Therapeutic upregulation of the ubiquitin-proteasome pathway to clear misfolded proteins is technically challenging, and aggregation-
Panel 1: Model systems of Huntington’s disease

Cell models
Cell lines are valuable for biochemical investigations but they might not recapitulate the cell biology of neurons. They can be used for transient, stable, or inducible expression strategies.

Primary neurons recapitulate many features of neurons in vivo. Co-cultures or mixed cultures can reproduce some cell interactions, though not all the complexities of neuronal circuits.

Induced pluripotent stem cells are currently being derived from patients with Huntington’s disease for study of disease pathogenesis and for therapeutic screening. A goal (eg, with the NINDS-funded Huntington’s disease induced pluripotent stem cell consortium) will be to investigate if affected individuals have mutant HTT-related phenotypes such as toxic effects or changes in cell metabolism.

Invertebrate models (Drosophila or Caenorhabditis elegans)
Disease models in invertebrates can display progressive behavioural changes and phenotypes such as toxic effects or changes in cell metabolism.

Mouse models
A major issue for understanding disease biology and developing therapies is the extent to which mice and other animal models of Huntington’s disease recapitulate disease pathogenesis and predict response to experimental treatments.

An index of the validity for mouse models is that intranuclear inclusions were discovered in the R6/2 mouse model before their discovery in human post-mortem brain. However, no mouse models present the same massive striatal neuronal degeneration seen in humans.

Mouse models expressing N-terminal fragments of HTT (eg, the exon-1 or 90 amino acid N-terminal fragment of the R6/2 model; the 71 amino acid fragment of the N171-82Q model; or the caspase 6 fragment or S86 amino acid N-terminal fragment of the N586-82Q model) seem to have the most robust and rapidly progressive phenotypes, including incoordination, hindlimb clasping when suspended by the tail, gait instability on rotorod apparatus, cognitive and other behavioural abnormalities, and weight loss, progressing to early death, and thus have frequently been used for therapeutic trials.

Mice overexpressing full-length HTT generally present more subtle phenotypes than those mentioned above but may have somewhat more selective neurodegeneration; models incorporating the entire HTT gene using transgenic insertion via BACs or YACs have been used for studies of pathogenesis. The BAC, YAC, and knock-in models are especially valuable for studies where the entire HTT protein is needed, such as studies of cleavage of full-length HTT, or studies of stages before overt behavioural and pathological phenotypes. However, since the phenotypes develop so slowly, these studies require substantial commitment of time and resources.

Behavioural tests need to be standardised to yield useful comparability across laboratories. High-field-strength micro MRI studies provide an automated and highly quantitative measure, which can be used to track progression in models of Huntington’s disease and could be useful for preclinical therapeutic trials (figures 6C and 6D)

Large mammalian models
Pigs, sheep, or monkeys could have advantages for study of behaviour and in tests of whether gene therapy agents—such as viral expression vectors or antisense nucleotides—can penetrate throughout all the relevant regions of brain, including cortex, subcortical white matter, and subcortical grey matter nuclei.

NINDS=National Institute of Neurological Disorders and Stroke. BAC=bacterial artificial chromosome. YAC=yeast artificial chromosome.

Prone proteins may be poor substrates for the ubiquitin-proteasome system. Autophagy and lysosomal clearance might be more tractable targets. Mutant HTT can interfere with target recognition and compromise autophagic clearance. Pharmacological activation of mTOR (mammalian target of rapamycin)-dependent autophagy with rapamycin attenuated the toxic effects of mutant HTT in fly and mouse models of Huntington’s disease. Small-molecule enhancers of autophagy—including mTOR-independent pathways—could be of benefit. Molecules such as trehalose, calpastatin, nicardipine, and minoxidil, although non-selective in their effects, might be of interest for further development.

Another strategy to clear mutant HTT is to enhance activity of molecular chaperones, which can promote refolding of misfolded proteins. Overexpression of one or both of the chaperones HSP104 and HSP27 can suppress mutant HTT-mediated neurotoxicity in mouse and rat models of Huntington’s disease. Targeting the regulators of the stress-induced chaperone response could be possible, thereby coordinately inducing many chaperones with complementary cytoprotective functions. Alternatively, a better understanding of the particular chaperones most relevant to clearance of mutant HTT might be useful, to achieve enhanced specificity.

Conformation and aggregation of HTT
The presence of an expanded polyglutamine repeat in the HTT protein (either full-length or truncated) causes a conformational change, which is believed to trigger a pathogenic cascade (figure 3). The structure of normal HTT exon 1 (with a polyglutamine chain of 17 residues), crystallised as a fusion with maltose-binding protein, has an N-terminal α helix, a flexible polyglutamine stretch that can adopt either an α-helical, random-coil, or an extended-loop conformation, and a polyproline helix. Uncertainty surrounds the structure of the toxic form of polyglutamine and nearby regions. The structure of exon 1 bound to the 3B3H10 antibody, which recognises the toxic form of polyglutamine, is composed of a compact hairpin most consistent with two β strands and a turn.

Findings of in-vitro studies have suggested that the toxic conformation includes a so-called compact β conformation, with short β strands interspersed with β turns so that the strands are held together in an antiparallel conformation by intramolecular (and intermolecular) hydrogen bonds. The presence of abnormally folded protein, which can aggregate and form fibrillar structures, highlights similarities between Huntington’s disease and other neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease, and prion disorders.

The mechanism of HTT aggregation is complex. Initial phases seem to be accelerated by hydrophobic interactions within an amphipathic α-helical structure of 17 amino acids at the N-terminus. These
hydrophobic interactions could be targeted by therapeutic interventions for Huntington’s disease. According to findings of antibody recognition and structural studies, mutant HTT could have many different conformations.69,75 Soluble, intermediate, mutant HTT species are more toxic to neurons than are large, visible, intracellular aggregates.

Does the toxic species consist of soluble misfolded monomer or small soluble oligomeric species, or a combination?72,76–78 Recent studies have highlighted the roles of oligomeric species, which could be formed in several ways, including via N-terminal interactions or direct polyglutamine interactions.79–81 These oligomeric species may not be on the pathway to inclusion formation.

Initial attempts to develop conformational therapeutics targeted production of large aggregates, as detected using a filter binding assay. A polyphenol was identified, (--)-epigallocatechin gallate (EGCG), which could act to decrease toxic forms of HTT.82 A small-molecule aggregation inhibitor (a sulfobenzoic acid derivative termed C2-8) showed a beneficial effect on behavioural phenotypes and striatal neuronal volume in the R6/2 mouse model of Huntington’s disease,83 although it had no effect on survival. Identification of the toxic HTT species will be crucial for therapeutic strategies that attempt to intervene within the pathway of conformational change and aggregation.

**Figure 4: Postulated intercellular pathogenesis of Huntington’s disease**

Mutant HTT causes decreased transport and release of corticostriatal BDNF. Increased stimulation of extrasynaptic glutamate receptors takes place, and reuptake of glutamate by glia is diminished, leading to excitotoxicity and enhanced susceptibility to metabolic toxic effects. Activated microglia produce increased inflammatory activity. Mutant HTT itself might also be transmitted cell to cell. 3HK=3-hydroxykynurenine. QUIN=quinolinic acid. KMO=kynurenine 3-monooxygenase. ROS=reactive oxygen species. TrkB=tyrosine kinase B receptor. NMDA=N-methyl-D-aspartic acid.

**Panel 2: Phenotypic versus target-based screening approaches**

**High-throughput screens with defined targets**

A current paradigm for discovery of drugs is to identify a molecular target, such as an enzyme or a receptor, undertake a high-throughput biochemical screen, and then test the positive compounds in models of Huntington’s disease.47 Unfortunately, only a few, well-validated, specific molecular targets exist.

For targets in which the structure is known, such as caspase 6, an alternative to high-throughput screening is fragment-based lead discovery48 or other structure-based methods.

**High-content screens with phenotypic assays**

Phenotypic assays, such as those for HTT cellular toxic effects,49–51 can be used to screen directly for small molecules that can ameliorate toxicity. For example, a PC12-inducible cell model of HTT toxic effects was screened with a few FDA-approved compounds, with positive agents followed up by testing in mice.52 Even without a defined molecular target, the ability to do medicinal chemistry and then rapid rescreening in cell models potentially makes therapeutic development possible.

A variant of this strategy is to use the phenotypic model for a small interfering RNA screen to identify molecular targets and then develop assays for more traditional, small-molecule library screens.

**Natural products**

A potentially powerful approach is to use natural extracts in screens (either assay-based or phenotypic) and then purify the active compound. This approach has renewed credibility with modern methods of purification and molecular analysis.53

FDA=US Food and Drug Administration.
Post-translational modifications of HTT

Post-translational modifications of HTT (figure 2) are vital early steps in modulating the protein’s toxic effects, and since many changes are probably mediated by enzymes, these molecules could be good therapeutic targets. A major post-translational modification is phosphorylation.84–86

Phosphorylation at threonine 3 influences toxicity, and phosphorylation at serines 13 and 16 has mostly protective effects in vivo.84 Phosphorylation by AKT—and probably other kinases—at serine 421 reduces toxic effects in cell-culture experiments, although this finding has not been confirmed in vivo.73–77 Several other phosphorylation sites of HTT have been identified. Most phosphorylation events seem to be protective,87–91 so to be therapeutic targets, they would need to be activated, or a specific phosphatase would need to be identified as a target for inhibition.

HTT can undergo palmitoylation at cysteine 214, enhancing membrane association. Expansion of the polyglutamine tract diminishes this modification, which then contributes to enhanced neuronal toxicity.20 We do not know whether specific activation of HTT palmitoylation is possible, because many other proteins in the cell undergo this lipid modification. HTT can also be acetylated at lysine 444, augmenting its clearance.92 Again, it is not clear if this process can be enhanced selectively.

The 17 amino acids at the N-terminus of HTT are especially susceptible to post-translational modification (figure 2), including phosphorylation, ubiquitination, and attachment of SUMO (small ubiquitin-like modifier).85 Modification with SUMO usually accompanies transcriptional repression, providing a potential link to gene transcription (see HTT and gene transcription). The recently reported interaction between HTT and Rhes,39 as noted above (see Principles of pathogenesis), could underlie regional specificity, might affect SUMO modification and aggregation of HTT, and may also relate to metabolism. However, much is still uncertain about these possibilities.

Proteolytic cleavage of HTT

Much evidence from biochemical, cell, and animal models of Huntington’s disease, and from study of post-mortem tissue from affected individuals, suggests that proteolytic cleavage of HTT could be key for disease pathogenesis,95–97 although unique roles for the full-length protein are also likely.98 Inclusions can be labelled with antibodies to epitopes near the N-terminus of HTT, but not epitopes located nearer the C-terminus, and data from western blot studies indicate that inclusions contain truncated HTT species, including the N-terminus of the protein.99 Alzheimer’s disease sets a precedent for the importance of truncation of pathogenic protein in disease pathogenesis. Specific cleavage of amyloid precursor protein is vital for generation of the amyloid β peptide,
and for Alzheimer’s disease pathogenesis, and this process has suggested major therapeutic strategies. Some cleavage events promote toxic effects of amyloid precursor protein, whereas others ameliorate the effects. Similarly, in Huntington’s disease, cleavage can take place at several places, generating fragments of various sizes with different properties; therefore, understanding the specific sites of HTT proteolysis will be important.

One cleavage site is predicted to involve caspase 6 cleavage sequence at position S86 (figure 2). Transgenic mice with yeast artificial chromosome (YAC) constructs expressing mutant HTT with alterations at the S86 position had strikingly less pathological effects than did control littermates. Two caveats are that alterations of this cleavage site could also change the conformation of HTT in the region, and that this experiment depends on the two YAC transgenic mouse models having equivalent expression levels in all relevant cell types. Studies are underway to cross YAC transgenic mice with caspase 6 knock-out mice, and these findings will be vital to define the role of caspase 6 cleavage in pathogenesis of Huntington’s disease. Specific caspase 6 inhibitors are in development. The structure of caspase 6 could provide a starting point for drug development.

In addition to HTT fragments cleaved by caspases, smaller fragments are detected in human post-mortem tissue and in mouse models, small fragments can be highly toxic in vivo, as seen in the R6/2 and N171-82Q mouse models. Some of these small fragments have been termed cp-1 (or cp-A) and cp-2 (or cp-B; figure 2). Findings of biochemical studies have suggested that cp-2 can be produced by cleavage at position 167 in cell models. Work done in mouse models expressing full-length HTT has suggested that many fragments are present, with a key fragment around the size of cp-1 entering the nucleus.

**HTT and gene transcription**

An important aspect of pathogenesis of Huntington’s disease is believed to entail alterations of gene transcription. In extensive gene expression array studies, gene expression patterns have been identified in Huntington’s disease models and human post-mortem brain tissue. Cell culture and biochemical studies indicate that mutant HTT can interfere with gene transcription. Several molecular mediators have been proposed, including CBP (cAMP response element binding protein), NCoR (nuclear receptor corepressor), SP1 transcription factor, basal transcription factors, and REST (repressor element 1 silencing transcription factor) elements. Direct HTT interaction with DNA might also play a part.

Alterations of gene transcription triggered by mutant HTT have stimulated a great deal of experimental therapeutic interventions. Part of the activity of transcriptional activators such as CBP includes acetylation of histones and opening up DNA for transcription. Opposing enzymes, termed histone deacetylases (HDACs), cause transcriptional repression. HDACs have been targets for treatment of cancer and, thus, small-molecule inhibitors are available. HDAC inhibitors—such as SAHA (suberoylanilide hydroxamic acid), phenylbutyrate, and pimelic diphenylamide—can ameliorate the Huntington’s disease phenotype in mouse and invertebrate models. Recent findings suggest that HDAC4 could be especially relevant, as genetically engineered R6/2 mice with reduced amounts of HDAC4 have extended survival, improvement of the motor phenotype, and associated upregulation of relevant genes. However, even if a drug can be proven to be a specific HDAC inhibitor, we should not assume that it works via alterations of gene transcription. For instance, inhibition of HDAC6 probably works via alterations in cell transport.

A target gene with reduced transcription in patients with Huntington’s disease is BDNF. Interventions such as serotonin-selective reuptake inhibitors, ampakines, HDAC inhibitors, and BDNF itself, which all act via different mechanisms to increase the amount of neurotrophic support, have beneficial effects in mouse models of Huntington’s disease, indicating a productive avenue for further therapeutic development. Findings have also implicated HTT in RNA metabolism. The extent to which the polyglutamine expansion alters this function is still not clear, and the relation to toxic effects has not yet been well studied, but this discovery could lead to additional targets.

**Vesicular trafficking and cytoskeleton signalling**

HTT regulates cytoskeletal motor functions, including vesicle transport and recycling, in part via interactions with HAP1, HAP40 (huntingtin-associated protein of 40 kDa), and dynene. The expansion mutation of HTT disrupts this transport, including vesicular trafficking of BDNF. Thus, restoration of BDNF activity could help to compensate for the effects of mutant HTT in both transcription and vesicular transport.

HTT has also been connected to calcium signalling via binding to the type 1 inositol trisphosphate receptor, suggesting that calcium regulation could potentially be a therapeutic target, for which several drugs are already available. Other signalling pathways, such as the JNK kinase pathway could also provide targets.

**Metabolism**

Mutant HTT could have effects on cellular metabolism in several different ways. First, the cell must deal with the unfolded and abnormal protein, via mechanisms (eg, the ubiquitin proteasome pathway, autophagy, chaperones) that require energy (figure 5). Second, mutant HTT could have direct or indirect effects on mitochondria (figure 3), compromising energy metabolism and increasing oxidative damage. Third, calorie restriction can ameliorate the Huntington’s disease phenotype in mouse models, indicating that pathways related to aging and cell
metabolism can modify the disease’s pathogenesis. Fourth, transcription of PPARGC1A (formerly PGC1A) is altered by mutant HTT. The encoded protein, PGC1α, is itself a transcription factor, which in turn controls transcription of many nuclear-encoded proteins necessary for mitochondrial function and cellular energy metabolism. Class III HDACs (sirtuins) can also regulate cellular metabolism and are potentially important targets, particularly if they have a role not only in regulation of longevity but also in specific aspects of cell metabolism relevant to Huntington’s disease. Resveratrol has beneficial effects in some models of the disease. It could act in part as a sirtuin activator, but it probably has many other effects that need to be elucidated.

Treatment strategies aimed at amelioration of the cellular energy deficit and improvement of mitochondrial function in Huntington’s disease could have several beneficial effects. Agents might include coenzyme Q10, creatine, or combinations of these substances, or other candidates such as rosiglitazone or exendin 4. Two major phase III trials in Huntington’s disease (2CARE and CREST-E) are testing coenzyme Q10 and creatine, respectively.

Excitotoxicity, inflammation, and the quinolinic acid pathway
Excitotoxicity (excessive stimulation of excitatory amino acid receptors, especially NMDA receptors) has long been postulated to be a non-cell-autonomous mechanism with a role in pathogenesis of Huntington’s disease. Antixcitotoxic drugs have been tested in clinical trials but have proved disappointing so far, with negative outcomes for riluzole and remacemide, although whether these were the best agents to test the hypothesis is not clear. Blockage of extrasynaptic rather than synaptic NMDA receptors (eg, with memantine) might be more effective. However, the therapeutic window could be very narrow.

Inflammatory proteins such as complement proteins and clusterin are upregulated both peripherally and in the brain in patients with Huntington’s disease. Findings of PET imaging, in-vitro work, and post-mortem studies have shown that microglia are activated in prodromal and manifest Huntington’s disease, and that microglial activation correlates with disease severity and striatal loss. Evidence of innate immune activation, such as increased cytokines, has been reported both centrally and peripherally in affected individuals and mouse models of Huntington’s disease, beginning in the prodromal period, suggesting that abnormal immune activation could have a role in disease pathogenesis. HTT toxic effects in yeast genetic models can be regulated by kynurenine 3-monoxygenase (KMO), which is a key microglial enzyme implicated in reactive oxygen species generation and excitotoxicity (figure 4). Drugs targeting the KMO pathway are in development, and data from Huntington’s disease mouse models treated with a novel KMO inhibitor or crossed to KMO knockout mice show prolonged survival and improved neuropathology. Inflammation is potentially a tractable target, especially peripheral inflammation. However it is presumably a fairly distal effect of mutant HTT, and ascertaining its effectiveness as a target needs further study.

Cell-replacement strategies
As Huntington’s disease advances, a late-stage intervention might be replacement of lost neurons. To date, small clinical trials have been undertaken with fetal donor tissue in the striatum. Unlike Parkinson’s disease, for which the goal is replacement of tonic secretion of dopamine by the nigrostriatal pathway, in Huntington’s disease, reconstitution of a functional dynamic information-processing circuit will be necessary—eg, cortex to medium spiny projection neurons of the striatum to the globus pallidus and the substantia nigra, then through the thalamus back to the cerebral cortex. One of the major challenges facing cell-replacement strategies will be to control differentiation of embryonic stem cells or induced pluripotent stem cells to specific neuronal phenotypes, such as medium spiny neurons, which encourage them to form functional circuits without ectopic connections, and ensure absence of aberrant growth or tumour formation. Furthermore, many other brain regions are important in Huntington’s disease, so this strategy’s likelihood of success is very unclear.

Development of outcomes and biomarkers for disease-modifying therapies
Over the past 10 years, many clinical trials in Huntington’s disease have been done. Up to now, no drug has proven efficacious in a randomised placebo-controlled trial of disease-modifying therapy. Clinical trials are challenging, because Huntington’s disease progresses slowly and there is clinical heterogeneity. The clinical rating scales used to assess progression, such as the unified Huntington’s disease rating scale, similar to all clinical rating scales, are subject to inter-rater and intrarater variability. Quantitative clinical biomarker assessments such as tongue force variability, metronome-guided tapping, grip force, and oculomotor assessments, and cognitive tests, are being developed.

The full penetrance of the HTT mutation and availability of predictive genetic testing affords an opportunity to attempt treatment during the prodromal period of Huntington’s disease. A major challenge is devising outcome measures for this period, during which, by definition, signs of manifest illness are not definitively present. A trial with motor onset as the only outcome measure could require thousands of participants. Therefore, we need to identify sensitive and stable biomarkers of change in patients with prodromal and early-stage Huntington’s disease (panel 3).
Blood biomarkers could be simplest but, so far, few results have been replicated consistently. One candidate is 8-OHdG, a marker of oxidative stress, which is increased in patients with manifest and prodromal Huntington’s disease, although this finding needs replication.

Neuroimaging methods have, so far, offered the best biomarkers during the prodromal period, and have the potential to provide correlations between mouse and human therapeutics (figure 6). Striatal atrophy is prominent early and continues steadily throughout the course of the prodrome and into the symptomatic period of Huntington’s disease. Other areas of the brain are also affected, including subcortical structures, and cortical thinning is widespread but heterogeneous. White-matter atrophy is striking in the prodromal period and in manifest disease. Understanding the extent and timing of white-matter change will be important for its use as a biomarker, but could also have considerable therapeutic implications, because if white matter is primary in the biology of Huntington’s disease, then gene therapy might need to target subcortical white matter as well as cortical and subcortical grey matter.

Functional imaging can also detect abnormalities in individuals during the Huntington’s disease prodrome and could even be sensitive enough to identify irregularities before detectable structural or behavioural changes. Magnetic resonance spectroscopy, especially with new high-field-strength magnets, might offer innovative opportunities for molecular biomarker identification, including lactate or other markers of cellular stress.

Findings of multicentre observational studies such as PREDICT-HD and TRACK-HD will enable identification of a panel of biomarkers that could be used as efficacy endpoints in future trials. In PREDICT-HD, researchers are following hundreds of US and Australian prodromal participants with detailed imaging, cognitive, blood, and other measures. The research team on TRACK-HD is following a smaller number of individuals in Europe and Canada, with similar measures, but the study includes early-stage patients and more frequent visits, with additional quantitative motor and oculomotor measures. A major challenge is to identify and validate biomarkers in these longitudinal studies, not only to evaluate their use as potential endpoints in a disease-modifying clinical trial but also to guide the nature and timing of therapeutic interventions. Of imaging measures so far, striatal volumes seem to have the best properties of early and progressive change, but subcortical white matter could have similar properties.

Conclusions and questions for future study
In almost 20 years since the gene mutation for Huntington’s disease was identified, important advances have been made, but much is still unknown, and fundamental questions remain.

Of all the protein interactions of mutant HTT, which are most important for pathogenesis? Which of the post-translational modifications of HTT will yield the best therapeutic targets? To what extent does loss of HTT function contribute to pathogenesis or modify the effects of gain of function? Therapeutic strategies focusing on mutant HTT expression—such as lowering HTT mRNA—seem promising, but we still do not know how much of a decline in normal or mutant HTT can be tolerated without cellular dysfunction or death.

Effects of mutant HTT that seem to be fairly distal, such as metabolic effects, might feed back to alter cellular ability to deal with misfolded proteins. Thus, we believe a good strategy is to be open-minded about which stages of pathogenesis to target. However, this raises the question, which of the many cellular pathways of pathogenesis will provide the most therapeutic benefit? Conversely, will augmentation of natural cellular pathways, such as the proteasome or autophagy-lysosome pathways, be possible without causing serious side-effects? Many questions relate to models and markers. Why do current animal models replicate so poorly the...
massive selective striatal cell death of human Huntington’s disease and what can be done to improve them? Enhanced disease models—both in-vivo and cellular models, such as induced pluripotent stem cells or other patient-derived cell models—will be vital.

What biomarkers will be most suitable for tracking disease progression and response to treatments, particularly in gene carriers who are clinically well? Presumably, specific pharmacodynamic biomarkers could exist for every class of drug but, ideally, markers of functional or neurochemical disease state would be available, which could be responsive to many neuroprotective drugs. We believe imaging markers hold great promise, but they need to be correlated with patient-related functional outcomes, and our methods of measuring such outcomes need improvement. Can we identify functional disease-related markers that will be responsive to therapeutics in the short term, or will we

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**Figure 6: MRI in patients with Huntington’s disease and mouse models**

(A) Voxel-based morphometry in human prodromal disease (PreA and PreB) indicates early changes in striatum and other brain regions including subcortical white matter compared with controls. As disease progresses (HD1 and HD2), striatal atrophy remains severe, but widespread brain atrophy arises, especially in other subcortical nuclei and subcortical white matter and in cortical grey matter. Red indicates substantial atrophy and yellow the greatest degree of atrophy. (B) Longitudinal striatal (caudate plus putamen) atrophy in human prodromal disease progresses steadily, as assessed both cross-sectionally and longitudinally. The three groups of patients (far from onset, mid, and near to predicted onset) were each divided into two subgroups (n=40–50). For all groups, the first point is striatal volume at the time of the first MRI scan, and the second point is volume at the second scan (about 2 years later). Error bars indicate SE. Replotted from ref 152 with permission of the BMJ Publishing Group. (C) In-vivo MRI images colour-coded by Jacobian maps to show atrophy in brains of R6/2 mice. Striatal atrophy is present but is not as selective as in patients with Huntington’s disease. The R6/2 model has an early and severe progressive phenotype. CPu=striatum. LV=lateral ventricles. Green, blue, and purple represent progressively greater atrophy, and yellow, red, and white represent progressively greater enlargement. Images courtesy of Wenzhen Duan. (D) Progressive striatal atrophy as detected by T2-weighted volumetric microMRI in the N171-82Q mouse model and quantified by Large Deformation Diffeomorphic Metric Mapping. The N171-82Q model also has a robust progressive phenotype, though beginning a little later than in the R6/2 model, so there could be more opportunity to observe a prodromal period. Substantial striatal atrophy was noted in 6-week-old Huntington’s disease mice (red line) compared with wild type controls (blue line), and as in human beings, the atrophy was progressive. N=8 mice. Modified from ref 30 with permission of Elsevier.
need to use structural imaging measures, which are fairly slow to change? Of course, we will not have formal validation of any of these directions until we receive both positive and definitive negative results from well-designed human therapeutic trials.

Huntington’s disease is perhaps the most amenable of the neurodegenerative diseases to early intervention, in view of its genetic predictability and ongoing biomarker studies of prodromal and manifest disease. Thus, research in Huntington’s disease may inform early-intervention strategies for other, more prevalent, neurodegenerative disorders such as Alzheimer’s disease or Parkinson’s disease. Huntington’s disease can be a model for neuroprotective drugs, with the possibility to delay or even prevent onset of manifest disease.

Contributors
CAR initiated the project, but otherwise both authors contributed equally to literature search, preparation of figures, and writing.

Conflicts of interest
CAR has been a consultant for or received honoraria from Vertex, iPierian, Zenobia, Lundbeck, and Merck (royalties for transgenic mice). SJT declares no conflicts of interest.

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