THERAPEUTICS DEVELOPMENT FOR TRIPLET REPEAT EXPANSION DISEASES

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Abstract | The underlying genetic mutations for many inherited neurodegenerative disorders have been identified in recent years. One frequent type of mutation is trinucleotide repeat expansion. Depending on the location of the repeat expansion, the mutation might result in a loss of function of the disease gene, a toxic gain of function or both. Disease gene identification has led to the development of model systems for investigating disease mechanisms and evaluating treatments. Examination of experimental findings reveals similarities in disease mechanisms as well as possibilities for treatment.

Simple sequence repeats occur throughout the human genome. In 1991 an expansion of one of these repeats, a trinucleotide (CAG) repeat in the gene that encodes the androgen receptor, was discovered in patients with a neurodegenerative disorder: spinal and bulbar muscular atrophy (SBMA)[1]. The repeat, which normally consists of 13–30 CAGs, lengthens to 40 or more CAGs in patients with this disease. Over the few years that followed, at least another 20 disorders were identified as trinucleotide repeat diseases[2] (TABLE 1). Disorders that have triplet repeat expansions in non-coding regions typically cause a loss of gene function or toxic effects at the mRNA level, whereas those that occur in coding regions result in an expanded polyglutamine or polyalanine tract in the protein product, which causes the protein to become toxic, with or without the loss of its normal function. Therefore, development of therapeutics for non-coding disorders has focused on restoring gene function or compensating for the loss of its function, whereas therapy for coding disorders is directed towards ameliorating the consequences of the toxic protein.

Although much progress has been made in understanding the molecular and cellular mechanisms that underlie each disease, effective treatments have not yet been developed. Nevertheless, the field is poised to develop new therapeutics in the coming years as a result of increasing insights into the pathophysiology of repeat expansion. Here we present current approaches to the development of therapeutics for repeat expansion disorders, with an emphasis on the most intensely studied disorders with preclinical and/or clinical data.

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Non-coding trinucleotide repeat disorders

Non-coding expanded repeats include CGG, GCC, GAA, CTG and CAG. The type of repeat and its location within the gene defines each disorder and its pathophysiology. Because these disorders might result from a loss of function, one approach to treatment is through gene replacement, although as more becomes known about the normal function of the relevant gene products, new targets for compensatory intervention are being identified.

Friedreich ataxia. Friedreich ataxia (FA) is the most common inherited ataxia (TABLE 1). Most patients have a homozygous GAA-repeat expansion within the first intron of the frataxin gene, which results in a deficiency of frataxin mRNA and protein. Frataxin levels are inversely correlated with the length of the GAA repeat in the shorter of the two alleles that are carried by the patient[3]. Loss of frataxin leads to accumulation of mitochondrial iron, increased

Table 1 | **Triplet repeat expansion disorders**

Disease	Symptoms	Gene	Locus	Protein
Non-coding repeats				
Friedreich ataxia	Ataxia, weakness, sensory loss	*FXN*	9q13–q21.1	Frataxin
Fragile X syndrome A	Mental retardation	*FMR1*	Xq27.3	Fragile X mental retardation 1 protein
Fragile X syndrome E	Mental retardation	*FMR2*	Xq28	Fragile X mental retardation 2 protein
Dystrophia myotonica 1	Weakness, myotonia	*DMPK*	19q13	Dystrophia myotonica protein kinase
Spinocerebellar ataxia 8	Ataxia	Antisense to *KLHL1*	13q21	Undetermined
Spinocerebellar ataxia 12	Ataxia	*PPP2R2B*	5q31–q33	Regulatory subunit of the protein phosphatase PP2A
Huntington disease-like 2	Chorea, dementia	*JPH3*	16q24.3	Junctophilin 3
Polyglutamine disorders				
Spinal and bulbar muscular atrophy	Weakness	*AR*	Xq13–q21	Androgen receptor
Huntington disease	Chorea, dementia	*IT15*	*4p16.3*	Huntingtin
Dentatorubral-pallidoluysian atrophy	Ataxia, myoclonic epilepsy, dementia	*DRPLA*	12p13.31	Atrophin 1
Spinocerebellar ataxia 1	Ataxia	*SCA1*	6p23	Ataxin 1
Spinocerebellar ataxia 2	Ataxia	*SCA2*	12q24.1	Ataxin 2
Spinocerebellar ataxia 3 (Machado–Joseph disease)	Ataxia	*SCA3/MJD*	14q32.1	Ataxin 3
Spinocerebellar ataxia 6	Ataxia	*CACNA1A*	19p13	α_{1A}-voltage-dependent calcium channel subunit
Spinocerebellar ataxia 7	Ataxia	*SCA7*	3p12–p13	Ataxin 7
Spinocerebellar ataxia 17	Ataxia	*TBP*	6q27	TATA box binding protein
Polyalanine disorders*				
Oculopharyngeal dystrophy	Weakness	*PABPN1*	14q11.2–q13	Poly(A)-binding protein 2
Congenital central hypoventilation syndrome	Respiratory difficulties	*PHOX2B*	4p12	Paired-like homeobox 2B
Infantile spasms	Mental retardation, epilepsy	*ARX*	Xp22.13	Aristaless-related homeobox, X-linked
Synpolydactyly	Limb malformation	*HOXD13*	2q31–q32	Homeobox D13

*Polyalanine expansions have also been reported among mutations in other genes, including *RUNX2* (runt-related transcription factor 2) in cleidocranial dysplasia, *ZIC2* (Zic family member 2) in holoprosencephaly, *HOXA13* (homeobox A13) in hand–foot–genital syndrome, and *FOXL2* (forkhead box L2) in type II blepharophimosis, ptosis, and epicanthus inversus syndrome. Small aspartic acid repeat expansions have been reported among other mutations in the *COMP* (cartilage oligomeric matrix protein) gene in patients with multiple epiphyseal dysplasia.

OPEN-LABEL TRIAL
A clinical trial in which both subjects and investigators know which drug is being tested and the doses that are being used.

DOUBLE-BLIND TRIAL
A study in which neither the investigator nor the subject know whether a medication or placebo is being used for any given subject, so as to prevent subjective bias on the part of the subject or investigator.

PLACEBO-CONTROLLED
The use of an inactive substance or treatment that seems to be the same as, and is given in the same way as, an active drug or treatment being tested. The effects of the active drug or treatment are compared with the effects of the placebo.

susceptibility to oxidative stress and a reduction in oxidative phosphorylation that is due to a deficiency of mitochondrial proteins that contain iron–sulphur clusters[4]. Although the primary function of frataxin is not completely clear, the cellular defects that result from its deficiency point to targets for intervention (FIG. 1).

It is likely that free-radical formation and oxidative stress underlie the pathophysiology of FA[5], and for this reason antioxidants have been investigated as a potential therapy. Idebenone, a short-chain analogue of coenzyme Q10 (ubiquinone), has been shown to decrease lipid peroxidation and enhance mitochondrial energy production[6]. Several clinical trials of idebenone have resulted in a reduction of cardiac hypertrophy in patients with FA, but without significant improvement in neurological function[7]. By selecting patients who are in the early stages of the disease and who are closer in age an OPEN-LABEL, 1-year trial did show improvement in neurological function[8]. These results indicate that idebenone might be a beneficial treatment for FA, although further DOUBLE-BLIND, PLACEBO-CONTROLLED studies that focus on quality of life and neurological outcomes are needed.

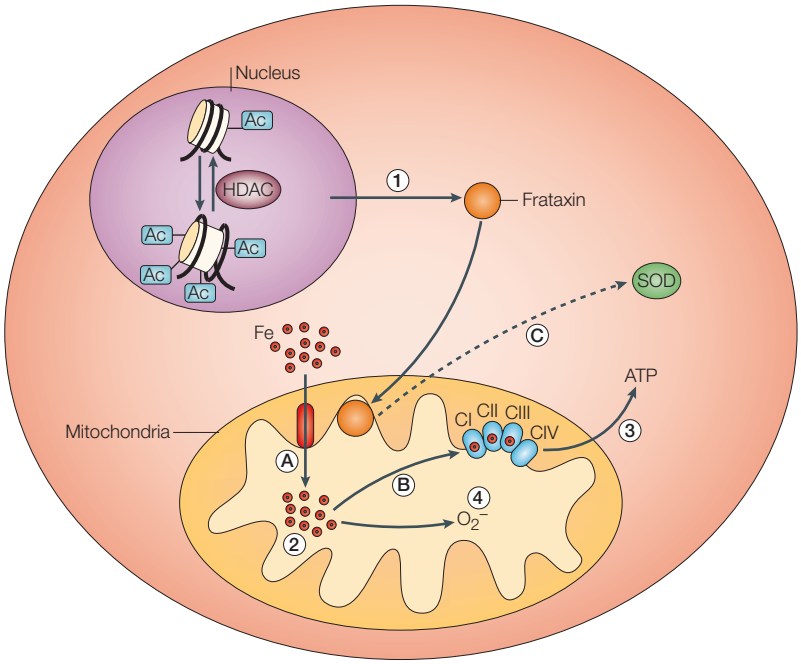

Figure 1 | **Friedreich ataxia: pathogenesis and points of intervention.** The expanded GAA repeat in the frataxin gene (*FXN*) results in reduced levels of the frataxin protein, which probably has a role in mitochondrial iron transport (**A**), iron–sulphur cluster assembly (**B**), and protection from free radicals (**C**), by increasing SOD activity. Consequently, therapeutic targets include enhancement of *FXN* transcription (**1**), selective mitochondrial iron chelation (**2**), enhancement of ATP generation to compensate for the lack of iron–sulphur proteins in the respiratory chain (**3**) and free-radical neutralization (**4**). Ac, acetylation; CI–CIV, class I–IV respiratory chain proteins; HDAC, histone deacetylase; SOD, superoxide dismutase.

Future clinical trials of idebenone in FA need to also consider drug dosing. Preliminary studies in FA and other diseases indicate a dose-related effect[9] and recent trials indicate that idebenone can be administered at much higher doses than those used previously, without dose-limiting toxicity (N.A.D., unpublished observations). On the basis of these findings, we are proceeding with a PHASE II CLINICAL TRIAL of high-dose idebenone in patients who have FA.

Further modification of the ubiquinone structure can lead to more potent antioxidants by enhancing their concentration in the mitochondria. For example, a lipophilic, cationic side chain on the ubiquinone derivative mitoquinone (MitoQ) allows it to accumulate within mitochondria[10], and it is substantially more potent than idebenone in protecting FA fibroblasts from oxidative stress[11]. MitoQ is currently being studied in PHASE I CLINICAL TRIALS.

Excess iron accumulation in mitochondria might have a role in FA pathogenesis, because free iron can enhance free-radical formation[12]. Therefore, iron-chelation therapy has also been considered as a potential therapeutic intervention. However, unless such chelation preferentially targets mitochondrial iron, normal cellular stores would be depleted. Pyridoxal isonicotinoyl hydrazone (PIH) mobilizes mitochondrial iron selectively[13]. A recently developed PIH-derivative shows promise as a potential therapeutic agent[14].

Despite being promising, antioxidant and chelation therapies do not address the primary biochemical defect in FA: frataxin deficiency. Recently, several compounds including cisplatin, haemin and sodium butyrate have been shown to enhance frataxin gene expression[15,16]. The mechanism of gene induction by these compounds is unclear, although sodium butyrate is known to induce gene expression by inhibiting histone deacetylation. Histone modulation has become an area of research interest for various diseases, including haemoglobinopathy, cancer and neurodegeneration, and might become more effective as precise histone deacetylase (HDAC) targets are identified.

Fragile X syndrome. Fragile X syndrome is caused by an expansion of a CGG repeat in the 5′ UTR of the fragile X mental retardation gene (*FMR1*). The resulting CpG hypermethylation at the FMR1 promoter[17] reduces *FMR1* transcription (FIG. 2).

Attempts have been made to reactivate *FMR1* transcription by removing the transcriptional blockade that is induced by excess promoter methylation. LYMPHOBLASTS from patients who have fragile X syndrome are treated *in vitro* with the DNA-demethylating agent 5-azadeoxycytidine (5-aza), and have increased levels of *FMR1* mRNA and protein[18]. However, there are two limitations to using a demethylation approach. First, long-term use of 5-aza is highly toxic, although less toxic compounds that have a similar mechanism of action might be developed in the future. Second, 5-aza must be incorporated into dividing cells to exert its effects, so it is not likely to be suitable for post-mitotic neurons. Nonetheless, other compounds might be able to reduce the methylation of the *FMR1* promoter. Fragile X lymphoblastoid cell lines that are exposed to L-carnitine or acetyl-L-carnitine show a mild decrease in *FMR1* promoter hypermethylation[19], but without increased transcription.

The inactive, hypermethylated *FMR1* gene is associated with hypoacetylated histones[20]. Treatment of lymphoblasts from fragile X patients with HDAC inhibitors has been reported to cause a modest increase in transcript levels, although not to the level seen with demethylating agents[21]. However, when HDAC inhibitors were used in combination with the demethylating agent 5-aza, transcript levels increased 2–5 fold compared with those obtained with 5-aza alone, which indicates that DNA methylation and histone deacetylation might work synergistically to silence genes, with methylation having a dominant role. Therefore, HDAC inhibitors might have a complementary adjunctive role in future therapy.

The downstream effects of FMR1 protein deficiency correlate with the clinical deficits in learning and memory. An *FMR1* knockout mouse has mild learning deficits and morphologically abnormal DENDRITES[22], which are similar to those seen in fragile X patients[23]. Abnormal electrophysiological properties of neurons in the knockout mouse are consistent with altered synaptic development and plasticity[24], and these have been linked to abnormal group 1 metabotropic glutamate receptor (mGluR) signalling, which primarily involves mGluR5.

PHASE II CLINICAL TRIAL
A study that is carried out to obtain more safety data and preliminary data on the effectiveness of the drug for a particular indication in patients with the disease or condition. Phase II studies help to determine the feasibility of larger-scale definitive trials.

PHASE I CLINICAL TRIAL
An initial clinical study that involves small numbers of healthy human volunteers and small doses to assess safety, metabolism and excretion of a drug.

LYMPHOBLASTS
Immature white blood cells.

DENDRITES
Short and typically highly branched extensions of the neuronal cell body that form synaptic contacts with the terminals of other neurons and allow the transmission of nerve impulses between cells.

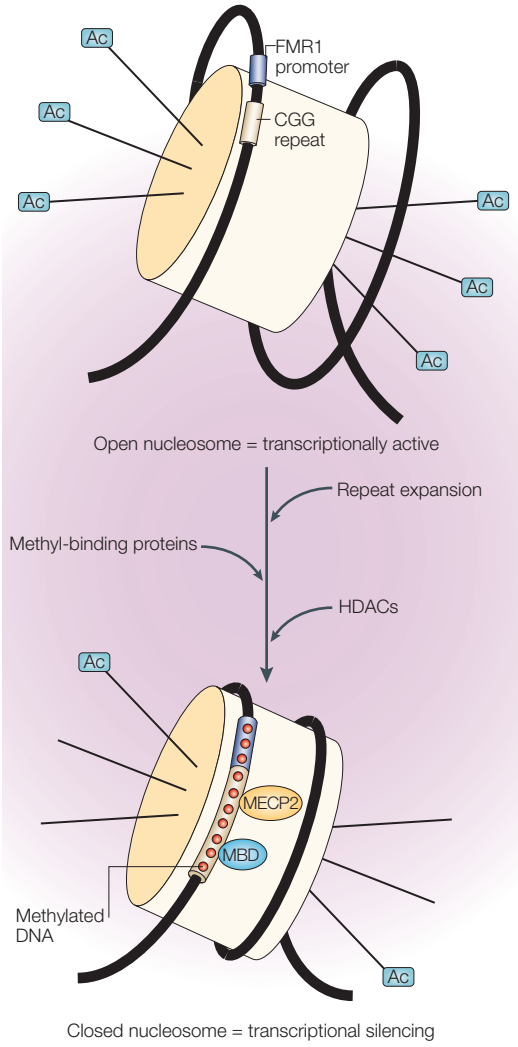

Figure 2 | **Promoter inactivation and transcriptional repression in fragile X syndrome.** The expansion of a CGG repeat in the 5′ UTR of the fragile X mental retardation 1 (*FMR1*) gene results in hypermethylation of the repeat region (upper panel), which recruits methyl-DNA-binding proteins, such as methyl-CpG-binding protein 2 (Rett syndrome) (MECP2) and methyl-CpG-binding domain protein (MBD) that repress transcription. These proteins also recruit histone-modifying enzymes, including histone deacetylases (HDACs), which further repress transcription (lower panel). Ac, acetylation.

It has been speculated that antagonists of this receptor might ameliorate the symptoms of fragile X syndrome[25]. Indeed, mGluR antagonists can correct neuroanatomical and behavioural defects in a *Drosophila* model of fragile X syndrome[26]. Overall, these compounds warrant further investigation as possible treatments for this disorder.

Myotonic dystrophy. Previous clinical trials for dystrophia myotonica 1 (DM1) have focused on symptomatic treatment to relieve either the MYOTONIA or the effects of muscle degeneration. Many drugs studied so far, including selenium and vitamin E, baclofen, nifedipine, creatine monohydrate and testosterone, have failed to demonstrate a definite beneficial effect (for a review see REF. 27). Other compounds such as phenytoin, carbamazepine, tocainide, impiramine and DHEA-S, had some clinical benefit, although these studies generally had few subjects and were not placebo-controlled, making the observations difficult to interpret (for a review see REF. 27).

The genetic mutation in DM1 is an expanded CTG trinucleotide repeat in the 3′ UTR of the protein kinase gene *DMPK* (dystrophia myotonica protein kinase)[28]. The expanded CTG repeat is unstable, with a tendency to increase in length with intergenerational transfer, resulting in a highly variable phenotype and ANTICIPATION. The CTG expanded transcript forms nuclear inclusions and sequesters specific RNA-binding proteins such as the muscleblind protein, which leads to a reduction in their activity[29]. Phylactou and colleagues have demonstrated the potential therapeutic effect of removing the expanded repeat region with *trans*-splicing group I intron ribozymes that modify the 3′ end of the *DMPK* transcript, both *in vitro* and *in vivo*[30]. Recently, pharmacotherapy that has a more immediate potential has been demonstrated in a new cell-based model of DM1. This cell line, which carries a 250-CTG repeat in the 3′ UTR of a luciferase reporter gene, shows gene effects in *cis* and cytotoxicity. A chemical compound library screen showed that DHEA-S and bioflavonoids are able to ameliorate both *cis*-effects on gene expression and cytotoxicity [31]. The exact mechanism that underlies these effects is unclear. Although it is known that flavonoids have potent antioxidant capacity, they also have various other biological activities[32]. Certain flavonoids can activate the class III, or sirtuin, family of HDACs[33] that has recently been shown to increase cellular stress defences and can ameliorate toxicity in models of Huntington disease (HD)[34]. It is therefore possible that, in this cell-based model system for DM1, flavonoids function by modulating epigenetic factors that are similar to those involved in other triplet repeat expansion disorders.

Polyglutamine expansion diseases

This family of disorders involves expansion of translated CAG repeats in the disease genes, resulting in expanded polyglutamine tracts in the respective proteins. Although the expanded polyglutamine tract itself can be toxic, it is in the context of the full-length protein that the distinctive selective neuronal loss occurs. This might be due to specific interactions that are mediated by the flanking regions[35–37]. Transgenic animal studies have supported a toxic gain-of-function mechanism that leads to neuronal dysfunction and death, although recent evidence indicates that loss of normal protein function might also have a role in disease pathogenesis[38,39]. Although the normal functions and pathological effects of the disease proteins are not completely clear, there seem to be common pathways that could be exploited in the development of therapeutics (FIG. 3).

Protein misfolding and aggregation. Aggregation of the disease protein is a feature that is shared between the polyglutamine disorders. Cleavage studies indicate that

MYOTONIA
The failure of muscle to relax immediately after voluntary contraction has stopped.

ANTICIPATION
The tendency of certain diseases to have an earlier age of onset and increasing severity in successive generations.

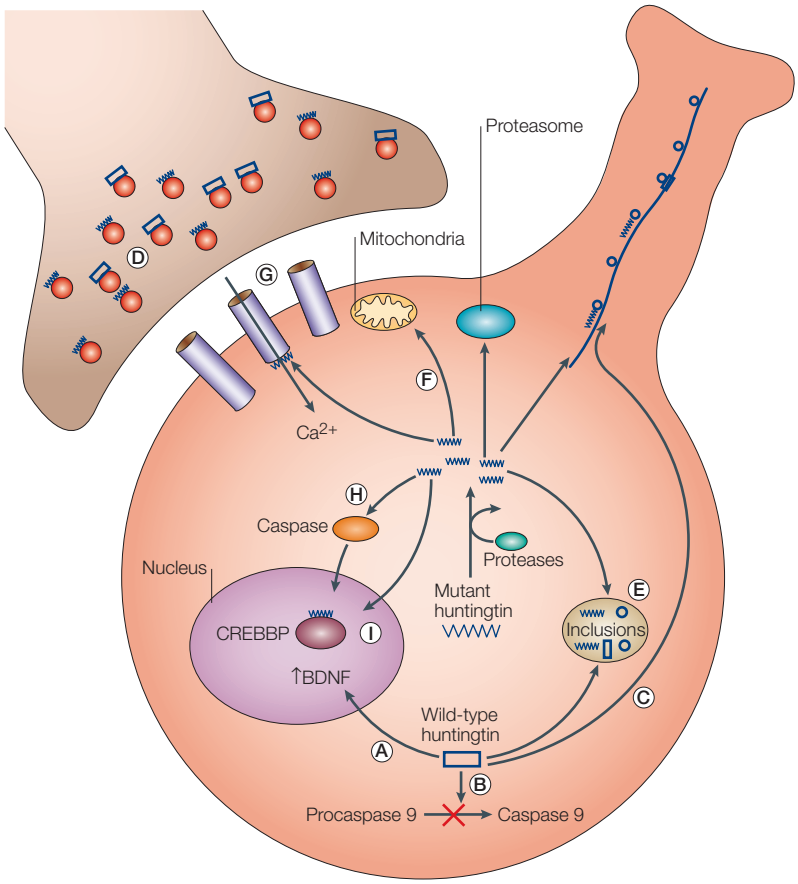

Figure 3 | **Mechanisms of toxicity in Huntington disease.** Wild-type huntingtin is important for normal cellular development and has been proposed to be involved in transcriptional regulation of brain-derived neurotrophic factor (BDNF) (**A**), prevention of procaspase 9 cleavage (**B**), and transport (**C**) and release (**D**) of vesicles. Mutant huntingtin is cleaved by proteases and might disrupt cellular processes by various mechanisms: sequestration of normal cellular proteins that include wild-type huntingtin (**E**), mitochondrial dysfunction and oxidative stress (**F**), altered neurotransmitter release and receptor function (**G**), activation of caspases that initiate the apoptotic cascade (**H**), and interactions with nuclear factors that result in transcriptional dysregulation (**I**). Many of these mechanisms have become targets for therapeutic intervention. CREBBP, CREB-binding protein.

the expanded polyglutamine tract imparts a new three-dimensional conformation to the mutated proteins. It is likely that the misfolded proteins acquire a toxic function through aberrant protein interactions. The mutant proteins are probably targeted for degradation, as they are often conjugated to ubiquitin, both in cell models and patient tissues[40,41]. When the cellular capacity to process the mutant proteins is exceeded, the proteins become clustered into insoluble protein aggregates. Inhibition of the PROTEASOME enhances aggregation, whereas overexpression of specific chaperone proteins that aid in protein folding reduces aggregate formation and toxicity[42,43]. In line with these observations, activation of the heat-shock response using the drug geldanamycin reduces aggregate formation in cell models[44].

Other compounds that have been reported to reduce polyglutamine aggregation and toxicity include benzothiazoles[45] and Congo red[46]. When Congo red was administered to an R6/2 mouse model of HD various aspects of the disease phenotype, including protein aggregation, were significantly reduced[47]. Trehalose is another compound that was found to prevent protein aggregation in a screening assay[48]. HD mice that were fed trehalose had significantly fewer inclusions in the CORTEX and STRIATUM and showed behavioural improvement.

Biological agents have also been shown to inhibit aggregation by binding to proposed interaction sites. Antibodies to the polyproline region of the mutant huntingtin protein blocked aggregation and cell death in a cell model[49]. Similarly, a bivalent huntingtin-binding peptide suppressed aggregation and polyglutamine toxicity in a *Drosophila* HD model[50].

Polyglutamine toxicity might be enhanced through crosslinking by transglutaminase[51,52]. Treatment of HD mice with cysteamine, a competitive transglutaminase inhibitor, reduced the severity of disease and increased survival[53]. However, the inclusions did not change histologically following treatment, which indicates that cysteamine might not function through transglutaminase inhibition, but rather by upregulating chaperone expression[54] or inhibiting caspases[55]. Recent genetic evidence supports a role for transglutaminase in polyglutamine toxicity that is independent of aggregate formation[56], which indicates that transglutaminase is still a viable target for intervention. Overall, inhibition of aggregate formation has been a reasonable screening target that has yielded some promising compounds worthy of further investigation.

Caspase activation and apoptosis. There is evidence of apoptosis (which involves caspase inactivation) in various neurodegenerative disorders, both *in vivo* and *in vitro*[57]. Several polyglutamine disease proteins are substrates for caspase cleavage[58,59]. Because truncation of polyglutamine proteins tends to increase cellular toxicity, blocking caspase activity might reduce toxicity both by reducing polyglutamine cleavage and by blocking apoptosis. Mutation of the caspase 3 cleavage site in the huntingtin protein reduces protein cleavage and cellular toxicity *in vitro*[60]. Likewise, crossing HD mice with transgenic mice that carry dominant–negative caspase 1 yielded double heterozygous transgenic offspring that had decreased cleavage of endogenous huntingtin and a decrease in the progression of the disease[61]. Disease progression was also slowed in HD mice that were exposed to the caspase inhibitor zVAD-fmk, a derivative of a synthetic tetrapeptide. More recently, it has been recognized that the antibiotic minocycline prevents activation of caspase 1 and caspase 3 during disease progression in an HD mouse model[62] and reduces the manifestations of the disease. In fact, minocycline might be broadly neuroprotective beyond its ability to reduce caspase activation[63]. A small clinical study indicated a benefit in patients with HD[64], supporting the need for future larger, double-blind, placebo-controlled trials.

Tauroursodeoxycholic acid (TUDCA) is a hydrophilic bile acid with apparent antioxidant, mitochondrial stabilization and anti-apoptotic activity. Treatment of transgenic HD mice with TUDCA reduced striatal atrophy and apoptosis, and improved the behavioural deficit[65].

PROTEASOME
A cytosolic protein complex that degrades proteins that have been marked for destruction by the ubiquitylation pathway.

CORTEX
The superficial layer of grey matter that is involved in higher functions, including initiation of voluntary movements, cognition and emotion.

STRIATUM
The region of the brain that receives excitatory input from the cortex, thalamus and midbrain. It has a pivotal role in modulating motor activity and higher cognitive function.

Because disease manifestations might precede cell loss, it is likely that apoptosis occurs late in the disease as a consequence of accumulated toxic effects. Nonetheless, caspases might serve as catalysts of apoptosis by cleaving polyglutamine proteins into toxic fragments, which in turn further increase caspase activation and therefore amplify the toxic cascade. Further studies should refine our understanding of this pathogenic mechanism and identify optimal targets for drug treatment.

Excitotoxicity and neurotransmission. Excitotoxicity has been proposed to have a role in various neurodegenerative disorders. Injections of excitatory amino-acid analogues into rodent brains have reproduced the histological and behavioural defects that are associated with HD[66,67]. Mutant huntingtin enhances neuronal vulnerability to receptor agonists[68,69]. In conjunction with enhanced vulnerability, neurons might also be exposed to elevated levels of neurotransmitters[70] as a result of the effects of mutant huntingtin on vesicular proteins, exocytosis and endocytosis[71]. Altered levels of neurotransmitter receptors have also been observed in the pre-symptomatic HD mouse brain[72]. It is not surprising then that various compounds have been examined for their ability to modulate the manifestations of the disease through receptor blockade or activation.

Several studies have focused on inhibiting glutamate and the *N*-methyl-D-aspartate (NMDA) receptor to prevent excitotoxicity in HD. Clinical trials in patients with HD using the NMDA-receptor antagonist amantadine found only minimal symptomatic benefit[73–75]. Trials with other NMDA antagonists including ketamine and baclofen also had limited success[76,77]. Another trial using the glutamate-release inhibitor lamotrigine reduced abnormal movements (chorea) in patients with HD, but had no effect on disease progression[78]. The drug riluzole, which inhibits glutamate release and might modulate signal-transduction pathways and inactivate voltage-gated sodium channels, also reduced chorea in patients with HD, but motor performance and overall function were not improved[79].

The limited clinical benefit found in these trials might be related to the complexity of NMDA-receptor physiology, kinetics and subunit composition. The NMDA receptor is a tetramer that consists of the subunits NR1, NR2A–D, and occasionally NR3A or B. The subunit composition determines the pharmacology and kinetics of the receptor-channel complex. The subunits are differentially expressed in different brain regions, with the NR2B subunit being predominantly expressed on medium spiny neurons, the most vulnerable population of neurons in HD. Activity in NR2B-containing NMDA receptors is potentiated by mutant huntingtin *in vitro*[80,81]. Recent findings that NR2A and NR2B might be genetic modifiers of the age of onset of HD[82] indicate that use of subunit-specific antagonists of NMDA might be a fruitful approach to therapy. However, to be clinically useful a receptor antagonist should maintain normal function while blocking excess activation. The difficulty of achieving this balance might account for the side effects that have been seen in clinical trials so far, and might be in part responsible for their failure. In essence, a drug that has low receptor affinity and only binds to open channels might yield the desired effect of reducing excitotoxicity while minimizing adverse events. The drug memantine has such a profile[83] and has recently been shown to slow down the progression of HD in an open-label, 2-year trial[84].

There is also evidence that receptors other than NMDA might contribute to neuronal damage. In addition to the ion-channel-gated glutamate receptors the mGluRs can contribute to cell toxicity through improper activation of membrane enzymes and second messenger systems[85]. The mGluR5 receptors are highly expressed by STRIATAL PROJECTION NEURONS and are involved in phosphoinositide hydrolysis, whereas the mGluR2 and 3 receptors serve as a negative-feedback control for glutamate release. Chronic administration of the mGluR2/3 agonist, LY379268, or the mGluR5 antagonist, 2-methyl-6-(phenylethynyl)-pyridine, to pre-symptomatic HD mice resulted in increased survival, decreased hyperactivity and mildly improved motor coordination[86]. In addition, blocking the A2A adenosine receptor and the cannabinoid CB1 receptor subtype have neuroprotective effects in chemically induced HD rodent models[87,88]. Overall, aberrant receptor activation seems to have a role in polyglutamine pathogenesis, and mitigating this process could enhance neuronal function and viability, and ultimately clinical outcomes.

Oxidative stress and neuroprotection. As with excitatory amino acids, infusion of mitochondrial toxins into rodent brains can cause selective striatal neurodegeneration[89]. This observation led to the hypothesis that mitochondrial dysfunction has a role in HD pathogenesis. Several lines of evidence indicate abnormal energy metabolism, including reduced glucose metabolism, elevated lactate levels and impaired mitochondrial-complex activity in patients with HD[90,91]. Defects in glucose metabolism reduce ATP synthesis and lead to the generation of free radicals and oxidative damage. Several agents that improve mitochondrial function or have an antioxidant activity have been tested in animal models. Various compounds, including ascorbate, BN8245, creatine, coenzyme Q10 with remacemide and α-lipoic acid, have been found to attenuate various aspects of the HD phenotype in transgenic mouse models[92]. However, most of these compounds were administered just before or at the onset of behavioural deficits, which indicates that these agents might be more effective in disease prevention rather than modifying disease manifestations at later stages. One exception was the creatine study, in which treatment later in the disease course also had some benefit[93]. Because of the safety and potential therapeutic value of creatine, a 1-year, double-blind, placebo-controlled study was carried out in 41 patients with HD[94]. Functional, neuromuscular and cognitive status did not change significantly after 1 year of creatine therapy, although the dosage used was substantially lower than that used in the previous animal studies, and the range of disease severity might have confounded overall analysis. A project that is funded by

STRIATAL PROJECTION NEURONS
These are medium sized, GABA-containing neurons of the striatum that project to the substantia nigra and have an important role in the regulation of movement.

the US National Institutes of Health is currently collecting pilot clinical data on the impact of creatine on HD symptoms and progression by examining various markers to help develop more sensitive and quantifiable indices for future PHASE III TRIALS.

In one of the largest trials so far, the Huntington Study Group (HSG) conducted a randomized, placebo-controlled study of coenzyme Q10 versus remacemide versus a combination of both in patients with relatively early-stage HD[95]. Although none of the treatments significantly changed the rate of decline in total functional capacity there was a trend towards slower disease progression among patients that were treated with coenzyme Q10. On the basis of these findings the HSG is currently conducting a trial to examine the safety and tolerability of higher doses of coenzyme Q10.

Part of the benefit of coenzyme Q10 therapy might be that it functions not only as an antioxidant, but also as an electron carrier to restore the ATP deficiency that occurs in HD. In this regard, compounds that have a similar structure and function to coenzyme Q10 might also be of clinical use — for example, idebenone. A 1-year, double-blind, placebo-controlled study using idebenone was conducted by the HSG, but no significant difference was seen between treated patients and controls in functional and neurological assessments[96]. However, this study had a small sample size and the dose of idebenone was relatively low (270 mg a day). Our recent results in patients with FA indicate that considerably higher idebenone doses can be tolerated (N.A.D. and K.H.F., unpublished observations), which indicates that, as with coenzyme Q10, high-dose idebenone treatment might be worthy of further testing.

Transcriptional dysregulation. Early changes in transcription occur in several polyglutamine disorders. Although some of these changes might be a response to perturbed cellular metabolism, proteins that have expanded polyglutamine tracts can directly interact with and sequester transcription factors. CREB-binding protein (CBP, also known as CREBBP), p300/CBP-associated factor (PCAF), $TAF_{II}130$ (also known as TBP-associated factor 4 (TAF4)) and SP1 (Sp1 transcription factor) have all been shown to interact with mutant polyglutamine proteins[97]. Overexpression of these transcription factors can overcome polyglutamine toxicity both *in vitro*[98,99] and *in vivo*[100], which indicates a role for transcriptional dysregulation in polyglutamine pathogenesis.

Several of the transcription factors that interact with mutant polyglutamine have acetyltransferase activity. Increased acetylation of histones relaxes the DNA structure around the nucleosome, thereby increasing transcription, whereas hypoacetylation represses gene activity[101]. Therefore, nuclear-factor sequestration by mutant polyglutamine proteins might dysregulate gene expression by altering the levels of histone acetylation. Expression of mutant huntingtin in cell culture reduces levels of acetylated histones H3 and H4 (REFS 102,103). Because the balance of histone acetylation is controlled by histone acetyltransferases and HDACs, sequestration and depletion of acetyltransferases might be corrected by HDAC inhibition. Treatment of *Drosophila* and mouse HD models with HDAC inhibitors has been shown to ameliorate the disease phenotype, with decreased cell degeneration and increased survival and motor performance associated with increased histone acetylation[102,104,105]. HDAC inhibition is an attractive clinical target as it has been investigated for other disorders that include cancer and haemoglobinopathy. HDAC inhibitors that are now in phase I and II clinical trials include phenylacetate, phenylbutyrate, valproic acid, suberoyl anilide hydroxamic acid, depsipeptide and MS-275. Many of these drugs might be limited in their application to triplet repeat disorders by their pharmacokinetic properties and/or side-effect profiles. However, two HDAC inhibitors, valproic acid and sodium phenylbutyrate, are currently FDA approved for treatment of other disorders, and the fact that their long-term use has limited toxicity[106,107] makes them potential clinical candidates.

There are 3 classes of HDACs, with a total of 17 members in humans, and it is possible that selective inhibition might be more effective than general inhibition at mitigating disease manifestations. There are several classes of compound with HDAC inhibitory activity[108], and developing new selective inhibitors is an active area of research. Activation of certain HDACs might also have a role in limiting toxicity. Overexpression of Sir2, a member of HDAC class III, extends the lifespan of various organisms, perhaps by deacetylating transcription factors of the FOXO (forkhead box, subclass O) family and thereby attenuating FOXO-mediated apoptosis[109]. Activation of the Sir2 gene family by the polyphenol RESVERATROL was shown to partially suppress the deleterious effects of mutant polyglutamine in a nematode and mouse neuronal culture model[34]. This could be a fruitful area for further research as there are several other activators and targets in this molecular pathway.

Trophic support, transplantation and selective inactivation. Neurotrophic factors contribute to neuronal growth and development and are released in response to various insults as an intrinsic mechanism of neuroprotection. Contrary to previous results, delivery of nerve growth factor (NGF) from genetically modified rodent fibroblasts *in vivo* can protect neurons from the effects of excitotoxins[110]. Subsequently, other neurotrophic factors, including ciliary neurotrophic factor (CNTF), glial-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF), have been delivered to chemically induced and transgenic HD models through infusion, viral vectors and engineered cell delivery, with varying degrees of efficacy[111–118]. Of the neurotrophic factors tested, BDNF is of particular interest: its production is regulated by huntingtin, and its levels are reduced in cultured cells and the cerebral cortex of transgenic HD mice and post-mortem HD brains[119].

Loss of neurotrophic factors could be specific to individual polyglutamine diseases. It has been shown that the mutant androgen receptor decreases CBP-mediated

PHASE III CLINICAL TRIAL
A study that is intended to gather the extra information about effectiveness and safety that is needed to evaluate the overall benefit–risk relationship of the drug. Phase III studies also provide a basis for extrapolating the results to the general population.

RESVERATROL
A natural compound that is found in grapes, mulberries, peanuts and other plants or food products, especially red wine, that has antioxidant, antimutagen and anti-inflammatory properties.

vascular endothelial growth factor (VEGF) transcription and that motor neuron cell death can be rescued by treatment with VEGF in a cell model of SBMA[120]. Although phase I clinical trials, including one in patients with HD[121], support the safety and feasibility of neurotrophin delivery, the choice of neurotrophin and the means of delivery need to be optimized, and long-term safety needs to be addressed.

Cell transplantation also offers the possibility of providing trophic support and restoration of neural circuitry, and therefore a substrate for functional improvement. Although fetal striatal grafting into transgenic HD models has had mixed results[122,123], several clinical trials have been undertaken. Eighteen months after transplantation of human fetal striatal tissue, post-mortem examination of one patient's brain showed that the graft contained neurons with appropriate connections[124]. There were no signs of inflammation or rejection, but clinical benefit was not evident. In another similar trial, cognitive and motor improvement was seen in three out of five patients, who also showed a partial restoration of metabolic activity, as measured by carrying out a PET SCAN[125,126], which indicates that the grafted neurons were not only active but functional. Some of the limitations of this approach relate to the number of cells that survive within the graft. Grafts of stem cells[127], including autologous bone marrow cells[128] and umbilical cord cells[129] that can be produced in a larger quantity, have demonstrated a benefit in rodent HD models and might warrant testing in clinical trials. Further investigation into the differentiation and integration of these cells is now needed. It will also be important to determine how they perform in relation to neighbouring populations of cells that might still be dysfunctional.

Because the mechanism of polyglutamine disease involves a toxic gain of function, another possible treatment is to inactivate the disease gene. Support for this approach comes from a study of a conditional HD mouse model in which decreased mutant huntingtin expression led to improvement in the behavioural and pathological phenotype[130]. Administering antisense RNAs against various CAG-repeat lengths reduced transcript levels and rescued toxicity in a mammalian cell-based model of SBMA[131]. More recently, introduction of an adeno-associated virus (AAV) expressing short hairpin RNAs into mouse models of spinocerebellar ataxia 1 (REF. 132) and HD[133] ameliorated motor and neuropathological abnormalities, which demonstrates the feasibility of such technology *in vivo*. However, whereas the target of gene silencing in transgenic mice was the exogenous transgene, such an approach in patients might affect the wild-type allele as well as the mutant one[38]. Identification of disease-linked polymorphisms might allow for allele-specific silencing and therefore maintain wild-type gene expression. This technique is appealing because it targets the underlying genetic defect. Although trophic support, transplantation and RNAi are all worthy of further investigation, they are still in early development and many technical issues remain to be resolved before clinical application.

PET SCAN
A positron emission tomography scan. This is an imaging technique that relies on the detection of γ-rays that are emitted from tissues after the administration of a natural biochemical substance into which positron-emitting isotopes have been incorporated.

Concluding remarks

Recent research in repeat expansion disease brings the goal of safe and effective treatment within sight. Although there are many obstacles to definitive therapy that would either restore normal gene function or selectively silence mutated ones, the tools are now available to develop treatments that could mitigate disease manifestations. Although the triplet repeat diseases are phenotypically diverse, there are common pathological mechanisms and common approaches to treatment, particularly for those disorders that are caused by polyglutamine expansion. Each of the diseases discussed here has an identified genetic defect and an underlying biochemical abnormality, which allows the development of assays for small molecule screening. A systematic approach for prioritizing candidate compounds that result from such screens is important. Secondary assays are needed to remove false positives and to identify compounds that have the desired effect; medicinal chemistry is needed to optimize efficacy; and judicious use of available animal models is needed for proof of concept and to verify safety and efficacy before proceeding with clinical testing.

It is useful to establish prospective guidelines in selecting compounds to move forward into clinical trials through evaluating and grading all preclinical data for each candidate, as has been proposed for Parkinson disease[134]. A similar analysis (SET-HD, Systematic Evaluation of Treatments for Huntington's Disease) is being carried out by the HSG to help identify drugs that are worthy of clinical study for HD in the short term. These guidelines help to direct the clinical investigator when choosing study treatments, bearing in mind the other factors that must also be considered in trial design, which include dose, pharmacokinetics, biomarkers, outcome measures, time of treatment relative to stage of disease and combination therapy. Studies that address these factors will increase the likelihood of success in developing treatments.

There are several drug candidates with reported efficacy across several model systems. However, the effects of single agents have been limited. It could be that combination therapies with agents that target different aspects of the disease mechanism will have better overall outcomes. There has been little preclinical evaluation of combination therapy for these disorders so far, and more systematic evaluation of such therapies is needed.

Overall, the neurological disorders that are caused by repeat expansion are now ready for the kind of systematic empirical and mechanism-based approaches that have been applied successfully to the development of new treatments for other diseases such as cancer and infectious diseases. The repeat expansion diseases might not be prevalent enough to attract significant pharmaceutical investment, but the defined targets that are represented by their genetic and biochemical defects, the availability of cell-culture and animal models, and the prospects for treatment that could be effective across multiple diseases make these disorders prime targets for the development of therapeutics. It is likely that progress made in this field will be carried over to benefit patients who have other neurological diseases in the future.

1. La Spada, A. R., Wilson, E. M., Lubahn, D. B., Harding, A. E. & Fischbeck, K. H. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* **352**, 77–79 (1991).
2. Cummings, C. J. & Zoghbi, H. Y. Fourteen and counting: unraveling trinucleotide repeat diseases. *Hum. Mol. Genet*. **9**, 909–916 (2000).
3. Campuzano, V. *et al.* Frataxin is reduced in Friedreich ataxia patients and is associated with mitochondrial membranes. *Hum. Mol. Genet*. **6**, 1771–1780 (1997).
4. Wilson, R. B. Frataxin and frataxin deficiency in Friedreich's ataxia. *J. Neurol. Sci.* **207**, 103–105 (2003).
5. Pandolfo, M. Molecular basis of Friedreich ataxia. *Mov. Disord.* **16**, 815–821 (2001).
6. Gillis, J. C., Benefield, P. & McTavish, D. Idebenone. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in age-related cognitive disorders. *Drugs Aging* **5**, 133–152 (1994).
7. Rotig, A., Sidi, D., Munnich, A. & Rustin, P. Molecular insights into Friedreich's ataxia and antioxidant-based therapies. *Trends Mol. Med.* **8**, 221–224 (2002).
This is a review of the consequences of frataxin loss and the rationale behind antioxidant therapy.
8. Artuch, R. *et al.* Friedreich's ataxia: idebenone treatment in early stage patients. *Neuropediatrics* **33**, 190–193 (2002).
9. Gutzmann, H. & Hadler, D. Sustained efficacy and safety of idebenone in the treatment of Alzheimer's disease: update on a 2-year double-blind multicentre study. *J. Neural Transm. Suppl.* **54**, 301–310 (1998).
10. Kelso, G. F. *et al.* Selective targeting of a redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties. *J. Biol. Chem.* **276**, 4588–4596 (2001).
11. Jauslin, M. L., Meier, T., Smith, R. A. & Murphy, M. P. Mitochondria-targeted antioxidants protect Friedreich ataxia fibroblasts from endogenous oxidative stress more effectively than untargeted antioxidants. *FASEB J.* **17**, 1972–1974 (2003).
12. Wardman, P. & Candeias, L. P. Fenton chemistry: an introduction. *Radiat. Res.* **145**, 523–531 (1996).
13. Ponka, P., Borova, J., Neuwirt, J., Fuchs, O. & Necas, E. A study of intracellular iron metabolism using pyridoxal isonicotinoyl hydrazone and other synthetic chelating agents. *Biochim. Biophys. Acta* **586**, 278–297 (1979).
14. Richardson, D. R., Mouralian, C., Ponka, P. & Becker, E. Development of potential iron chelators for the treatment of Friedreich's ataxia: ligands that mobilize mitochondrial iron. *Biochim. Biophys. Acta* **1536**, 133–140 (2001).
15. Sarsero, J. P. *et al.* Upregulation of expression from the *FRDA* genomic locus for the therapy of Friedreich ataxia. *J. Gene Med.* **5**, 72–81 (2003).
16. Ghazizadeh, M. Cisplatin may induce frataxin expression. *J. Nippon Med. Sch.* **70**, 367–371 (2003).
17. Pieretti, M. *et al.* Absence of expression of the *FMR-1* gene in fragile X syndrome. *Cell* **66**, 817–822 (1991).
18. Chiurazzi, P., Pomponi, M. G., Willemsen, R., Oostra, B. A. & Neri, G. *In vitro* reactivation of the *FMR1* gene involved in fragile X syndrome. *Hum. Mol. Genet.* **7**, 109–113 (1998).
19. Pascale, E. *et al.* Modulation of methylation in the FMR1 promoter region after long term treatment with L-carnitine and acetyl-L-carnitine. *J. Med. Genet.* **40**, e76 (2003).
20. Coffee, B., Zhang, F., Warren, S. T. & Reines, D. Acetylated histones are associated with *FMR1* in normal but not fragile X-syndrome cells. *Nature Genet.* **22**, 98–101 (1999).
21. Chiurazzi, P. *et al.* Synergistic effect of histone hyperacetylation and DNA demethylation in the reactivation of the *FMR1* gene. *Hum. Mol. Genet.* **8**, 2317–2323 (1999).
22. Nimchinsky, E. A., Oberlander, A. M. & Svoboda, K. Abnormal development of dendritic spines in *FMR1* knock-out mice. *J. Neurosci*. **21**, 5139–5146 (2001).
23. Irwin, S. A. *et al.* Dendritic spine and dendritic field characteristics of layer V pyramidal neurons in the visual cortex of fragile-X knockout mice. *Am. J. Med. Genet.* **111**, 140–146 (2002).
24. Huber, K. M., Gallagher, S. M., Warren, S. T. & Bear, M. F. Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc. Natl Acad. Sci. USA* **99**, 7746–7750 (2002).
25. Bear, M. F., Huber, K. M. & Warren, S. T. The mGluR theory of fragile X mental retardation. *Trends Neurosci.* **27**, 370–377 (2004).
The theory of excessive metabotropic receptor function in fragile X syndrome.
26. McBride, S. M. *et al.* Pharmacological rescue of synaptic plasticity, courtship behavior, and mushroom body defects in a *Drosophila* model of fragile X syndrome. *Neuron* **45**, 753–764 (2005).
27. Meola, G. & Sansone, V. Treatment in myotonia and periodic paralysis. *Rev. Neurol.* **160**, S55–S69 (2004).
28. Fu, Y. H. *et al.* An unstable triplet repeat in a gene related to myotonic muscular dystrophy. *Science* **255**, 1256–1258 (1992).
29. Timchenko, N. A. *et al.* RNA CUG repeats sequester CUGBP1 and alter protein levels and activity of CUGBP1. *J. Biol. Chem.* **276**, 7820–7826 (2001).
30. Phylactou, L. A., Darrah, C. & Wood, M. J. Ribozyme-mediated trans-splicing of a trinucleotide repeat. *Nature Genet.* **18**, 378–381 (1998).
31. Furuya, H. *et al.* Some flavonoids and DHEA-S prevent the *cis*-effect of expanded CTG repeats in a stable PC12 cell transformant. *Biochem. Pharmacol.* **69**, 503–516 (2005).
32. Scalbert, A., Johnson, I. T. & Saltmarsh, M. Polyphenols: antioxidants and beyond. *Am. J. Clin. Nutr.* **81**, S215–S217 (2005).
33. Howitz, K. T. *et al.* Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature* **425**, 191–196 (2003).
34. Parker, J. A. *et al.* Resveratrol rescues mutant polyglutamine cytotoxicity in nematode and mammalian neurons. *Nature Genet.* **37**, 349–350 (2005).
This paper describes HDAC (sirtuin family) activation as a means of suppressing polyglutamine toxicity.
35. Reddy, P. H. *et al.* Behavioural abnormalities and selective neuronal loss in HD transgenic mice expressing mutated full-length HD cDNA. *Nature Genet*. **20**, 198–202 (1998).
36. Sato, T. *et al.* Transgenic mice harboring a full-length human mutant *DRPLA* gene exhibit age-dependent intergenerational and somatic instabilities of CAG repeats comparable with those in DRPLA patients. *Hum. Mol. Genet*. **8**, 99–106 (1999).
37. Katsuno, M. *et al.* Testosterone reduction prevents phenotypic expression in a transgenic mouse model of spinal and bulbar muscular atrophy. *Neuron* **35**, 843–854 (2002).
The authors describe the ligand-dependent toxicity of the mutant androgen receptor in Kennedy disease. This emphasizes the need to understand each polyglutamine disorder in the context of the disease protein's normal function.
38. Reiner, A., Dragatsis, I., Zeitlin, S. & Goldowitz, D. Wild-type huntingtin plays a role in brain development and neuronal survival. *Mol. Neurobiol.* **28**, 259–276 (2003).
39. Cattaneo, E. Dysfunction of wild-type huntingtin in Huntington disease. *News Physiol. Sci*. **18**, 34–37 (2003).
40. Cummings, C. J. *et al.* Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1. *Nature Genet.* **19**, 148–154 (1998).
41. Chai, Y., Koppenhafer, S. L., Shoesmith, S. J., Perez, M. K. & Paulson, H. L. Evidence for proteasome involvement in polyglutamine disease: localization to nuclear inclusions in SCA3/MJD and suppression of polyglutamine aggregation *in vitro*. *Hum. Mol. Genet.* **8**, 673–682 (1999).
42. Kazemi-Esfarjani, P. & Benzer, S. Genetic suppression of polyglutamine toxicity in *Drosophila*. *Science* **287**, 1837–1840 (2000).
43. Wyttenbach, A. *et al.* Effects of heat shock, heat shock protein 40 (HDJ-2), and proteasome inhibition on protein aggregation in cellular models of Huntington's disease. *Proc. Natl Acad. Sci. USA* **97**, 2898–2903 (2000).
44. Sittler, A. *et al.* Geldanamycin activates a heat shock response and inhibits huntingtin aggregation in a cell culture model of Huntington's disease. *Hum. Mol. Genet.* **10**, 1307–1315 (2001).
45. Heiser, V. *et al.* Identification of benzothiazoles as potential polyglutamine aggregation inhibitors of Huntington's disease by using an automated filter retardation assay. *Proc. Natl Acad. Sci. USA* **99**, 16400–16406 (2002).
This paper describes the application of high-throughput screening for polyglutamine disorders and shows that inhibitors of aggregation are therapeutic targets.
46. Smith, D. L. *et al.* Inhibition of polyglutamine aggregation in R6/2 HD brain slices-complex dose-response profiles. *Neurobiol. Dis.* **8**, 1017–1026 (2001).
47. Sanchez, I., Mahlke, C. & Yuan, J. Pivotal role of oligomerization in expanded polyglutamine neurodegenerative disorders. *Nature* **421**, 373–379 (2003).
48. Tanaka, M. *et al.* Trehalose alleviates polyglutamine-mediated pathology in a mouse model of Huntington disease. *Nature Med.* **10**, 148–154 (2004).
The authors discuss an approach to inhibiting protein aggregation using a saccharide–polyglutamine interaction. The safety profile of this chemical seems exceptionally favourable.
49. Khoshnan, A., Ko, J. & Patterson, P. H. Effects of intracellular expression of anti-huntingtin antibodies of various specificities on mutant huntingtin aggregation and toxicity. *Proc. Natl Acad. Sci. USA* **99**, 1002–1007 (2002).
50. Kazantsev, A. *et al.* A bivalent Huntingtin binding peptide suppresses polyglutamine aggregation and pathogenesis in *Drosophila*. *Nature Genet.* **30**, 367–376 (2002).
51. Kahlem, P., Terre, C., Green, H. & Djian, P. Peptides containing glutamine repeats as substrates for transglutaminase-catalyzed cross-linking: relevance to diseases of the nervous system. *Proc. Natl Acad. Sci. USA* **93**, 14580–14585 (1996).
52. Karpuj, M. V. *et al.* Transglutaminase aggregates huntingtin into nonamyloidogenic polymers, and its enzymatic activity increases in Huntington's disease brain nuclei. *Proc. Natl Acad. Sci. USA* **96**, 7388–7393 (1999).
53. Dedeoglu, A. *et al.* Therapeutic effects of cystamine in a murine model of Huntington's disease. *J. Neurosci.* **22**, 8942–8950 (2002).
54. Karpuj, M. V. *et al.* Prolonged survival and decreased abnormal movements in transgenic model of Huntington disease, with administration of the transglutaminase inhibitor cystamine. *Nature Med.* **8**, 143–149 (2002).
This article provides an example of effective treatment after the development of disease manifestations. It also describes activity of the drug outside its pharmacological target.
55. Lesort, M., Lee, M., Tucholski, J. & Johnson, G. V. Cystamine inhibits caspase activity. Implications for the treatment of polyglutamine disorders. *J. Biol. Chem.* **278**, 3825–3830 (2003).
56. Bailey, C. D. & Johnson, G. V. Tissue transglutaminase contributes to disease progression in the R6/2 Huntington's disease mouse model via aggregate-independent mechanisms. *J. Neurochem.* **92**, 83–92 (2005).
57. Wellington, C. L. & Hayden, M. R. Caspases and neurodegeneration: on the cutting edge of new therapeutic approaches. *Clin. Genet.* **57**, 1–10 (2000).
58. Goldberg, Y. P. *et al.* Cleavage of huntingtin by apopain, a proapoptotic cysteine protease, is modulated by the polyglutamine tract. *Nature Genet.* **13**, 442–449 (1996).
59. Wellington, C. L. *et al.* Caspase cleavage of gene products associated with triplet expansion disorders generates truncated fragments containing the polyglutamine tract. *J. Biol. Chem.* **273**, 9158–9167 (1998).
60. Wellington, C. L. *et al.* Inhibiting caspase cleavage of huntingtin reduces toxicity and aggregate formation in neuronal and nonneuronal cells. *J. Biol. Chem.* **275**, 19831–19838 (2000).
61. Ona, V. O. *et al.* Inhibition of caspase-1 slows disease progression in a mouse model of Huntington's disease. *Nature* **399**, 263–267 (1999).
62. Chen, M. *et al.* Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. *Nature Med.* **6**, 797–801 (2000).
The authors describe the amelioration of disease manifestations in an HD model with a drug that inhibits caspase.
63. Wang, X. *et al.* Minocycline inhibits caspase-independent and -dependent mitochondrial cell death pathways in models of Huntington's disease. *Proc. Natl Acad. Sci. USA* **100**, 10483–10487 (2003).
64. Bonelli, R. M., Hodl, A. K., Hofmann, P. & Kapfhammer, H. P. Neuroprotection in Huntington's disease: a 2-year study on minocycline. *Int. Clin. Psychopharmacol.* **19**, 337–342 (2004).
65. Keene, C. D. *et al.* Tauroursodeoxycholic acid, a bile acid, is neuroprotective in a transgenic animal model of Huntington's disease. *Proc. Natl Acad. Sci. USA* **99**, 10671–10676 (2002).
66. Coyle, J. T. & Schwarcz, R. Lesion of striatal neurones with kainic acid provides a model for Huntington's chorea. *Nature* **263**, 244–246 (1976).
67. Beal, M. F. *et al.* Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. *Nature* **321**, 168–171 (1986).
68. Cepeda, C. *et al.* NMDA receptor function in mouse models of Huntington disease. *J. Neurosci. Res.* **66**, 525–539 (2001).
69. Zeron, M. M. *et al.* Increased sensitivity to *N*-methyl-D-aspartate receptor-mediated excitotoxicity in a mouse model of Huntington's disease. *Neuron* **33**, 849–860 (2002).
70. Nicniocaill, B., Haraldsson, B., Hansson, O., O'Connor, W. T. & Brundin, P. Altered striatal amino acid neurotransmitter release monitored using microdialysis in R6/1 Huntington transgenic mice. *Eur. J. Neurosci.* **13**, 206–210 (2001).

71. Li, J. Y., Plomann, M. & Brundin, P. Huntington's disease: a synaptopathy? *Trends Mol. Med.* **9**, 414–420 (2003).
72. Cha, J. H. *et al.* Altered neurotransmitter receptor expression in transgenic mouse models of Huntington's disease. *Phil. Trans. R. Soc. Lond. B* **354**, 981–989 (1999).
73. O'Suilleabhain, P. & Dewey, R. B. Jr. A randomized trial of amantadine in Huntington disease. *Arch. Neurol.* **60**, 996–998 (2003).
74. Lucetti, C. *et al.* Amantadine in Huntington's disease: open-label video-blinded study. *Neurol. Sci.* **23**, S83–S84 (2002).
75. Verhagen Metman, L. *et al.* Huntington's disease: a randomized, controlled trial using the NMDA-antagonist amantadine. *Neurology* **59**, 694–699 (2002).
76. Murman, D. L. *et al.* Cognitive, behavioral, and motor effects of the NMDA antagonist ketamine in Huntington's disease. *Neurology* **49**, 153–161 (1997).
77. Shoulson, I. *et al.* A controlled clinical trial of baclofen as protective therapy in early Huntington's disease. *Ann. Neurol.* **25**, 252–259 (1989).
78. Kremer, B. *et al.* Influence of lamotrigine on progression of early Huntington disease: a randomized clinical trial. *Neurology* **53**, 1000–1011 (1999).
79. Seppi, K. *et al.* Riluzole in Huntington's disease (HD): an open label study with one year follow up. *J. Neurol.* **248**, 866–869 (2001).
80. Li, L. *et al.* Role of NR2B-type NMDA receptors in selective neurodegeneration in Huntington disease. *Neurobiol. Aging* **24**, 1113–1121 (2003).
81. Li, L., Murphy, T. H., Hayden, M. R. & Raymond, L. A. Enhanced striatal NR2B-containing *N*-methyl-D-aspartate receptor-mediated synaptic currents in a mouse model of Huntington disease. *J. Neurophysiol.* **92**, 2738–2746 (2004).
82. Arning, L. *et al. NR2A* and *NR2B* receptor gene variations modify age of onset in Huntington's disease. *Neurogenetics* **6**, 25–28 (2005).
83. Chen, H. S. & Lipton, S. A. Mechanism of memantine block of NMDA-activated channels in rat retinal ganglion cells: uncompetitive antagonism. *J. Physiol.* **499**, 27–46 (1997).
84. Beister, A. *et al.* The *N*-methyl-D-aspartate antagonist memantine retards progression of Huntington's disease. *J. Neural Transm. Suppl.* **68**, S117–S122 (2004).
85. Nicoletti, F. *et al.* Group-I metabotropic glutamate receptors: hypotheses to explain their dual role in neurotoxicity and neuroprotection. *Neuropharmacology* **38**, 1477–1484 (1999).
86. Schiefer, J. *et al.* The metabotropic glutamate receptor 5 antagonist MPEP and the mGluR2 agonist LY379268 modify disease progression in a transgenic mouse model of Huntington's disease. *Brain Res.* **1019**, 246–254 (2004).
87. Popoli, P. *et al.* Blockade of striatal adenosine A_{2A} receptor reduces, through a presynaptic mechanism, quinolinic acid-induced excitotoxicity: possible relevance to neuroprotective interventions in neurodegenerative diseases of the striatum. *J. Neurosci.* **22**, 1967–1975 (2002).
88. Lastres-Becker, I. *et al.* Effects of cannabinoids in the rat model of Huntington's disease generated by an intrastriatal injection of malonate. *Neuroreport* **14**, 813–816 (2003).
89. Borlongan, C. V. *et al.* Systemic 3-nitropropionic acid: behavioral deficits and striatal damage in adult rats. *Brain Res. Bull.* **36**, 549–556 (1995).
90. Gu, M. *et al.* Mitochondrial defect in Huntington's disease caudate nucleus. *Ann. Neurol.* **39**, 385–389 (1996).
91. Browne, S. E. *et al.* Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. *Ann. Neurol.* **41**, 646–653 (1997).
92. Beal, M. F. & Ferrante, R. J. Experimental therapeutics in transgenic mouse models of Huntington's disease. *Nature Rev. Neurosci.* **5**, 373–384 (2004).
A review of the pathogenesis of HD in the context of mouse models and the efficacy of therapeutic agents tested in these models.
93. Dedeoglu, A. *et al.* Creatine therapy provides neuroprotection after onset of clinical symptoms in Huntington's disease transgenic mice. *J. Neurochem.* **85**, 1359–1367 (2003).
94. Verbessem, P. *et al.* Creatine supplementation in Huntington's disease: a placebo-controlled pilot trial. *Neurology* **61**, 925–930 (2003).
95. Huntington Study Group. A randomized, placebo-controlled trial of coenzyme Q10 and remacemide in Huntington's disease. *Neurology* **57**, 397–404 (2001).
96. Ranen, N. G. *et al.* A controlled trial of idebenone in Huntington's disease. *Mov. Disord.* **11**, 549–554 (1996).
97. Okazawa, H. Polyglutamine diseases: a transcription disorder? *Cell. Mol. Life Sci.* **60**, 1427–1439 (2003).
98. McCampbell, A. *et al.* CREB-binding protein sequestration by expanded polyglutamine. *Hum. Mol. Genet.* **9**, 2197–2202 (2000).
99. Dunah, A. W. *et al.* Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. *Science* **296**, 2238–2243 (2002).
100. Taylor, J. P. *et al.* Aberrant histone acetylation, altered transcription, and retinal degeneration in a *Drosophila* model of polyglutamine disease are rescued by CREB-binding protein. *Genes Dev.* **17**, 1463–1468 (2003).
101. Grewal, S. I. & Moazed, D. Heterochromatin and epigenetic control of gene expression. *Science* **301**, 798–802 (2003).
102. Steffan, J. S. *et al.* Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in *Drosophila*. *Nature* **413**, 739–743 (2001).
This is a description of reduced acetyltransferase activity and histone acetylation in polyglutamine disease, and how this can be overcome by HDAC inhibitors with consequent amelioration of toxicity.
103. Igarashi, S. *et al.* Inducible PC12 cell model of Huntington's disease shows toxicity and decreased histone acetylation. *Neuroreport* **14**, 565–568 (2003).
104. Hockly, E. *et al.* Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor, ameliorates motor deficits in a mouse model of Huntington's disease. *Proc. Natl Acad. Sci. USA* **100**, 2041–2046 (2003).
105. Ferrante, R. J. *et al.* Histone deacetylase inhibition by sodium butyrate chemotherapy ameliorates the neurodegenerative phenotype in Huntington's disease mice. *J. Neurosci.* **23**, 9418–9427 (2003).
This article discusses the beneficial effects of HDAC inhibitors in an HD mouse model.
106. Gottlicher, M. Valproic acid: an old drug newly discovered as inhibitor of histone deacetylases. *Ann. Hematol.* **83** (Suppl. 1), 91–92 (2004).
107. Burlina, A. B., Ogier, H., Korall, H. & Trefz, F. K. Long-term treatment with sodium phenylbutyrate in ornithine transcar bamylase-deficient patients. *Mol. Genet. Metab.* **72**, 351–355 (2001).
108. Kelly, W. K., O'Connor, O. A. & Marks, P. A. Histone deacetylase inhibitors: from target to clinical trials. *Expert Opin. Investig. Drugs* **11**, 1695–1713 (2002).
109. Brunet, A. *et al.* Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* **303**, 2011–2015 (2004).
110. Emerich, D. F. *et al.* Implants of polymer-encapsulated human NGF-secreting cells in the nonhuman primate: rescue and sprouting of degenerating cholinergic basal forebrain neurons. *J. Comp. Neurol.* **349**, 148–164 (1994).
111. Emerich, D. F. *et al.* Implants of encapsulated human CNTF-producing fibroblasts prevent behavioral deficits and striatal degeneration in a rodent model of Huntington's disease. *J. Neurosci.* **16**, 5168–5181 (1996).
112. Bemelmans, A. P. *et al.* Brain-derived neurotrophic factor-mediated protection of striatal neurons in an excitotoxic rat model of Huntington's disease, as demonstrated by adenoviral gene transfer. *Hum. Gene Ther.* **10**, 2987–2997 (1999).
113. Perez-Navarro, E., Canudas, A. M., Akerund, P., Alberch, J. & Arenas, E. Brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4/5 prevent the death of striatal projection neurons in a rodent model of Huntington's disease. *J. Neurochem.* **75**, 2190–2199 (2000).
114. McBride, J. L. *et al.* Structural and functional neuroprotection in a rat model of Huntington's disease by viral gene transfer of GDNF. *Exp. Neurol.* **181**, 213–223 (2003).
115. Kells, A. P. *et al.* AAV-mediated gene delivery of BDNF or GDNF is neuroprotective in a model of Huntington disease. *Mol. Ther.* **9**, 682–688 (2004).
116. Canals, J. M. *et al.* Brain-derived neurotrophic factor regulates the onset and severity of motor dysfunction associated with enkephalinergic neuronal degeneration in Huntington's disease. *J. Neurosci.* **24**, 7727–7739 (2004).
117. Cepeda, C. *et al.* Increased GABAergic function in mouse models of Huntington's disease: reversal by BDNF. *J. Neurosci. Res.* **78**, 855–867 (2004).
118. Popovic, N., Maingay, M., Kirik, D. & Brundin, P. Lentiviral gene delivery of GDNF into the striatum of R6/2 Huntington mice fails to attenuate behavioral and neuropathological changes. *Exp. Neurol.* **193**, 65–74 (2005).
119. Zuccato, C. *et al.* Loss of huntingtin-mediated *BDNF* gene transcription in Huntington's disease. *Science* **293**, 493–498 (2001).
120. Sopher, B. L. *et al.* Androgen receptor YAC transgenic mice recapitulate SBMA motor neuronopathy and implicate VEGF164 in the motor neuron degeneration. *Neuron* **41**, 687–699 (2004).
121. Bloch, J. *et al.* Neuroprotective gene therapy for Huntington's disease, using polymer-encapsulated cells engineered to secrete human ciliary neurotrophic factor: results of a phase I study. *Hum. Gene Ther.* **15**, 968–975 (2004).
122. Dunnett, S. B. *et al.* Striatal transplantation in a transgenic mouse model of Huntington's disease. *Exp. Neurol.* **154**, 31–40 (1998).
123. van Dellen, A., Deacon, R., York, D., Blakemore, C. & Hannan, A. J. Anterior cingulate cortical transplantation in transgenic Huntington's disease mice. *Brain Res. Bull.* **56**, 313–318 (2001).
124. Freeman, T. B. *et al.* Transplanted fetal striatum in Huntington's disease: phenotypic development and lack of pathology. *Proc. Natl Acad. Sci. USA* **97**, 13877–13882 (2000).
125. Bachoud-Levi, A. C. *et al.* Motor and cognitive improvements in patients with Huntington's disease after neural transplantation. *Lancet* **356**, 1975–1979 (2000).
126. Gaura, V. *et al.* Striatal neural grafting improves cortical metabolism in Huntington's disease patients. *Brain* **127**, 65–72 (2004).
127. McBride, J. L. *et al.* Human neural stem cell transplants improve motor function in a rat model of Huntington's disease. *J. Comp. Neurol.* **475**, 211–219 (2004).
128. Lescaudron, L., Unni, D. & Dunbar, G. L. Autologous adult bone marrow stem cell transplantation in an animal model of huntington's disease: behavioral and morphological outcomes. *Int. J. Neurosci.* **113**, 945–956 (2003).
129. Ende, N. & Chen, R. Human umbilical cord blood cells ameliorate Huntington's disease in transgenic mice. *J. Med.* **32**, 231–240 (2001).
130. Yamamoto, A., Lucas, J. J. & Hen, R. Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. *Cell* **101**, 57–66 (2000).
The authors show that blockade of expression of the mutant protein leads to a disappearance of inclusions and a reversal of the behavioural phenotype. This indicates that continuous expression of the mutant protein is necessary for the disease.
131. Caplen, N. J. *et al.* Rescue of polyglutamine-mediated cytotoxicity by double-stranded RNA-mediated RNA interference. *Hum. Mol. Genet.* **11**, 175–184 (2002).
132. Xia, H. *et al.* RNAi suppresses polyglutamine-induced neurodegeneration in a model of spinocerebellar ataxia. *Nature Med.* **10**, 816–820 (2004).
A discussion of *in vivo* use of RNAi as a potential therapy for dominant neurodegenerative disorders.
133. Harper, S. Q. *et al.* RNA interference improves motor and neuropathological abnormalities in a Huntington's disease mouse model. *Proc. Natl Acad. Sci. USA* **102**, 5820–5825 (2005).
This paper shows that RNAi can accomplish disease allele-specific silencing *in vivo*.
134. Ravina, B. M. *et al.* Neuroprotective agents for clinical trials in Parkinson's disease: a systematic assessment. *Neurology* **60**, 1234–1240 (2003).

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Competing interests statement

The authors declare no competing financial interests.

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