A bird's eye view on cellular dynamics in Huntington's disease

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Huntington's chorea is a rare neurodegenerative disorder caused by the dominant inheritance of the mutated huntingtin gene, housing poly-CAG or polyglutamine repeats. It is characterized by a wide variety of symptoms ranging from chorea and hypokinetic movements to behavioural and cognitive decline, followed by dementia and inevitable death. Over the past 120 years, all available therapeutics have been for the symptomatic management of Huntington's disease (HD) and require supportive physiotherapy and counselling to maximize the efficacy of the treatment. Several animal models have been employed to help elucidate and decrypt the pathophysiology of the disease, and also screen potential therapeutic candidates. In the last few decades, a deeper understanding of the cellular and molecular dynamics associated with HD has helped shed light on the mechanisms involved in disease progression. Genetic intervention for early detection, spreading awareness about HD and its symptoms, and training professionals in the nuances of the disease condition can significantly improve the lifestyle of patients. This article aims at summarizing the complex pathogenesis of HD at the cellular level using various disease models and available therapeutics.

Keywords: Cellular dynamics, excitotoxicity, gene therapy, mitochondrial dysregulation, neurodegeneration.

HUNTINGTON'S chorea is a heritable neurodegenerative disease characterized by distinct symptoms such as chorea and incoordination[1]. Owing to its detailed elucidation and annotation to the American physician George Huntington, the disease as well as its mutant protein have gained their distinctive name from him. The manifestation of an expanded poly-glutamine repeat chain at the N-terminal of the mutant protein has proven to be a hallmark in the identification of Huntington's disease (HD)[1,2].

Initial symptoms such as chorea are followed by imbalances in mental health leading to sadness and depression, drawing parallels to other neurodegenerative diseases[2]. As the disease progresses, a variety of motor, emotional, behavioural and cognitive symptoms are experienced, including unsteadiness, changes in sleeping patterns, delusions and hallucinations, intellectual decline and memory loss[3]. Thus, the vast spectra of symptoms that occur as the disease progresses make its pathology even more complex, requiring molecular and genetic intervention in early disease identification.

A famous study done in 1987 classified the disease into five grades (0–4) based on the degree of severity in the patients. Macroscopic and microscopic evaluation of 163 patients revealed the following standard classification protocol for HD patient diagnosis[4]. This meta-analysis laid the groundwork for future studies by connecting the dots between structural changes and their corresponding phenotypic changes in HD patients.

The caudate nucleus and putamen are regions of the brain that suffer from severe atrophy, as seen in late-stage HD patients' brains. The medium spiny neurons (MSN) located in the striatum employ the chemical γ-aminobutyric as a neurotransmitter and degenerate due to overexcitation in HD patients[5].

Originating from the short arm of chromosome 4p16.3, the Huntingtin protein (Htt) is a 348 kDa multi-domain protein that contains a polymorphic glutamine/proline-rich domain at its amino terminus corresponding to its CAG repeats[6]. These CAG repeats are referred to as a polyQ (polyglutamine) chain and disease severity corresponds to the length of this chain[6]. It is mainly cytoplasmic and is attached to the membrane via palmitoylation at cysteine 214 residue[7]. The protein is ubiquitously expressed, and its exact function still remains unknown; however, it has been associated with the healthy development and functioning of the nervous system[8]. Structurally, Htt is largely α-helical with its N- and C-terminal domains arranged in a solenoid fashion. These domains are connected by a smaller bridge domain, encompassing different types of tandem repeats[9].

The mutation in the Huntingtin protein is dynamic in nature[10]. This indicates that the degree of complication, as well as the age of onset and rate of worsening of symptoms, are all proportional to the number of CAG repeats[10]. Studies consistently show that repeats 7–35 correspond to a healthy phenotype, whereas surpassing a threshold of 40 repeats can result in mild to severe disease manifestations[11]. The average age of onset is considered to be around 40–60 years[11]. Owing to the phenomenon of genetic anticipation, prolonged accumulation of CAG repeats over generations precipitates a condition known as juvenile Huntington's disease, with an age onset of less than 20 years[12].

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The expanded polyQ chain enables a toxic gain of function by the mutant protein, as further corroborated by studies on animal models[13]. Details on the impact of gain of function are elaborated in detail in the following sections. However, knock-out and knock-down studies have shown that polyQ expansion within Htt can amount to a loss of function[14]. There is a loss of neuroprotective functions in the brain due to mutant protein blocking BDNF (brain-derived neurotrophic factor) increasing cellular toxicity[15]. The dual nature of the proteins is mainly due to higher mutant proteins stimulating cell stress and a decrease in healthy proteins that carry out neuronal homeostasis.

Extensive studies done on this complex pathological condition over many years have indicated over-activation or suppression of several different cellular mechanisms that gradually and sequentially weaken the cells, making the disease difficult to treat and manage with time. Events taking place in the cells are as follows: (1) The propensity of mutant Htt to take up abnormal confirmations, especially after post-translational modifications, leads to the formation of large β-sheet structures. This paves the way for abnormal accumulation of cytoplasmic aggregates. (2) Since management of these mutant aggregates puts additional stress on the cells, over time it leads to impairment of the normal cellular protein regulation machinery. (3) Translocation of these mutants to the nucleus enhances the virulence of the protein by stimulating transcriptional dysregulation. (4) Accumulation of reactive oxygen species (ROS) and a surplus of free Ca^{2+} ions in the cytoplasm due to mitochondrial and endoplasmic reticulum (ER) dysregulation decreases ATP production and hinders appropriate protein folding. (5) Excess production and uptake of glutamate cause overstimulation of neurons (excitotoxicity) with irregular post-synaptic signalling[12,16].

In this article, HD pathogenesis is discussed in detail, in the context of reported cellular and molecular manifestations. We also provide insight into the developments in the field using various animal models as well as clinical studies aimed at improving therapy.

HD prevalence, detection and diagnosis

The frequency of incidence of Huntington's chorea is about one in 100,000 Africans and Asians, and five in 100,000 Caucasians; thus, producing a significantly high occurrence rate among genetic diseases[1]. Zielonka *et al.*[17] have studied the gender differences observed in HD and reported a statistically significant bias in disease severity and rate of progression between men and women. Information is critical to enhancing patient care and therapy[17].

In 2004, the American College of Medical Genetics and Genomics (ACMG) Committee published an article to provide technique-specific guidelines to laboratories testing for HD. This article paved the way to establishing more standardized and reliable diagnostic testing guidelines for the US and non-US clinical laboratories in compliance with statutory regulations. It discusses the important points to consider in PCR detection with regards to patient controls, reference standards, presence of CCG polymorphism as well as Southern blot analysis, and care to be taken while interpreting the results[18].

The majority of disease diagnoses are neurological and motor-related using a genetic test for the HD CAG expansion or a confirmed family history of HD. Asymptomatic patients housing the mutant genotype are known as prodromal patients[19]. However, there is increasing prominence in diagnosis through cognitive and behavioural impairments, mainly because cognitive impairments are evident at least 15 years prior to when the motor diagnosis can be done[20]. Another large, internationally conducted longitudinal study 'PREDICT-HD' on 738 patients not yet diagnosed with HD sought to predict the number of years of onset of the disease based on neurocognitive characteristics[21].

Klöppel *et al.*[22] used multivariate support vector machine algorithms to predict the onset of HD in pre-symptomatic HD gene mutation carriers (PSCs) with or without prior information. It gave predictions with 83% accuracy in those patients, specifying at least a 33% chance of developing unequivocal signs of HD in five years based on prior information[22]. Imaging of the brain to identify biomarkers is another approach to diagnosis. Diffusion tensor imaging (DTI) has exposed anomalies in the orientation of neuronal fibre, which compromise the integrity of white matter and subcortical grey matter. Functional MRI (fMRI) is another tool that has the potential to recognise changes in the brain that premanifest HD even before standard structural brain damage occurs. PET scans, although expensive, are being used to analyse unique patterns seen in abnormal brain function in the prodromal stages of HD[23].

Molecular mechanisms in HD

The progression of HD occurs sequentially, as mentioned earlier and involves gradual interlinked changes over a patient's lifetime, which are described below in detail.

Dysregulated cellular protein management and accumulation of toxic aggregates

To effectively clear toxic/unwanted proteins from the cellular environment, non-lysosomal protein degradation processes such as the ubiquitin–proteasome pathway (UPP) are activated[24]. Research across the globe has shown a direct relationship between the increase in polyQ repeats and the quantum of proteasomes recruited by mutant Htt (mHtt) aggregates as well as the rate of aggregate formation in mouse models[24,25]. Reports also indicate an altered cell-wide proteasomal activity in the affected neurons due to the redistribution of proteolytic enzymes towards mHtt aggregates impairing whole-cell protein management[25,26].

The long poly-glutamate tail of the mutant protein has shown the ability to trap the Hsp70 and Hsp40 families of chaperones seen in both animal and cellular models of HD[27]. Sequestering of chaperones in aggregates reduces the level of soluble chaperones available in the cells, affecting the protein homeostasis of the cell. Although it has been shown that Hsp40 and Hsp70 act synergistically in suppressing neurotoxicity and increasing the solubility of mutant polyglutamine protein in *Drosophila* models[27], studies done to quantify the levels of these chaperones over the period of neurodegeneration in mice models have shown a progressive decrease of about 40% of the endogenous levels of chaperone proteins[28]. Sophisticated techniques using organotypic slice culture assays show that the overexpression of Hsp70 in mice models leads to an early but only transient decrease in aggregate formation[28]. Thus, the mutant protein evades cellular heat-shock responses while maintaining high aggregate levels that interfere with nuclear and cytoplasmic pathways.

Several studies have shown the involvement of caspases in the pathology of HD[29–32]. It has been shown that the presence of caspase 2-3 and -6 cleavage sites in mHtt and their ability to activate caspase-8 allow the mutant to manipulate caspase proteins in the cells[29]. Cleavage of proteins by caspase-3 produces toxic N-terminal fragments. Caspase inhibitors have been shown to attenuate cell death by reducing the quantum of noxious fragments produced and the rate of aggregate formation in apoptotic cells[30]. Furthermore, the caspase inhibitors Z-VAD-fmk and minocycline have been shown to slow down the progression of HD in transgenic mice. Based on this understanding, it has been proposed that targeting caspases may help slow down mutant protein aggregates[31,32].

Another key protein family identified is the calpain family of proteins. In 3-nitropropionic acid-induced animal models of HD, activation of calpains was found to contribute to the degeneration of striatal neurons[33]. Kim *et al.*[34] reported that calpain-mediated cleavage of Huntingtin produced two N-terminal huntingtin fragments, viz. 72 and 47 kDa, which were either smaller or approximately in the same size range as those produced by caspase-mediated cleavage. mHtt has been consistently proven to be more easily cleaved by calpain than caspases. These fragments easily enter the nucleus to form intra-nuclear inclusions[34]. Blockade of calpain-mediated cleavage of huntingtin reduces huntingtin aggregates and toxicity[35].

As the cells age, these and many other protective mechanisms and stress responses get activated. This further worsen the conditions by increasing mutant aggregate formation and aggravating neuronal loss in HD.

Impaired mitochondrial dynamics and reactive oxygen species

A hallmark of HD is the degeneration of medium spiny neurons (MSNs) in the striatum. Although the exact cause is unknown, mitochondrial dysfunction and loss of normal cellular bioenergetics might be critical in the accumulation of fragmented mitochondria[36]. Indicators of mitochondrial defects include decreased ATP levels, free-radical production, respiratory complex inhibition, loss of mitochondrial membrane potential, and Ca^{2+} buffering disturbances that culminate in targeting MSNs[37,38].

Fission and fusion of the mitochondria are normal active processes regulating cellular mitochondrial dynamics. They require large, evolutionary conserved GTPases that physically alter mitochondrial membranes and regulate their interaction with the organelles[39]. Fusion is carried out by membrane-expressed mitofusin 1, 2 (Mfn1, 2) and optic atrophy 1 (Opa1) that tether nearby mitochondria facilitating fusion, whereas proteins like dynamin-related protein 1 (Drp-1) and Fis1 (fission) regulate fission[40,41]. Studies have shown that abnormal mitochondrial dynamics are linked to interactions of mHtt with Drp-1 fission protein as well as an increase in cellular Drp1 levels and reduced Mfn1 levels that induce higher levels of fission and toxic enzymatic activity[28,42]. In the PGC-1 (peroxisome proliferator-activated receptor (PPAR)-γ coactivator) family of coactivators, PGC-1α is widely regarded as a master controller of mitochondrial biogenesis, playing a major role in mitochondrial protein transcriptional regulation[43]. It has been shown that mutant Huntingtin causes a reduction in TORC1, the most potent transcriptional activator of PGC-1α (ref. 44). Mice that had PGC-1α knocked-out and mutant huntingtin knock-in showed poor motor performance and earlier degeneration of the striatum than only mutant huntingtin knock-in mice, as well as increased susceptibility to the mitochondrial toxin 3-NP (ref. 43). Interference of mutant proteins with PGC-1α has been reported to cause disruption of oligodendrocytes and white matter as well as mitochondrial biogenesis impairment in muscle cells, leading to myopathy and exercise intolerance[45,46].

Reddy *et al.*[47] have shown that impaired mitochondrial function leads to high levels of ROS that include superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), as well as reactive nitrogen species such as peroxynitrite ($ONOO^-$) and $NO^{\bullet}$ that impair cellular function by degrading proteins, lipids and nucleic acids. This is consistent with studies showing that the addition of hydrogen peroxide to cultured cerebellar granule neurons induced mitochondrial fragmentation within 1 h of treatment of the cells[48]. It has been shown that acute exposure to relatively high levels of ROS, especially in the presence of calcium, can induce mitochondrial permeability, uncouple oxidative phosphorylation with catastrophic effects on mitochondrial energetics, and contribute to cytotoxicity via necrosis and/or apoptosis[49].

Mitochondria isolated from both the neuroblastoma and clonal striatal cells confirmed the interaction of mHtt with the outer mitochondrial membrane. This proved to directly increase the susceptibility of mitochondria to the calcium-induced MPT (mitochondria permeability transition) pore and the release of cytochrome *c* activating apoptotic pathways[50].

In summary, oxidative damage in neurons has, over the decades, been one of the central causative agents for several neurodegenerative diseases and has been implemented in exacerbating HD as well.

ER stress

Over the last decade, there have been several studies on the role of mHtt protein in producing ER stress, which affects a plethora of cellular functions. Converging evidence highlights the effect of the protein on the secretory pathway, including perturbations at the level of ERAD/protein quality control, ER–Golgi trafficking, endocytosis, vesicular trafficking, ER calcium homeostasis and autophagy/lysosomal-mediated protein degradation[51].

ER-associated degradation (ERAD) machinery includes chaperones, transmembrane proteins and ubiquitin-associated enzymes that select, target and retro-translocate misfolded proteins to the cytoplasm for degradation by the proteasome system, which is now dysregulated[52,53]. The mutant protein interfers with these pathways associated with the ERAD machinery leading to ER stress[53].

The unfolded protein response pathway (UPR) regulates and promotes adaptive processes in case of ER stress to recover cellular homeostasis and maintain normal protein folding mechanisms. IRE1α (inositol-requiring enzyme1), a stress sensor, controls the upregulation of a subset of UPR-related genes, including genes linked to folding, protein quality control, the ERAD system, and ER–Golgi biogenesis. IRE1α also interacts with proteins to activate the apoptosis signal-regulating kinase 1 (ASK1) and c-Jun-N terminal kinase (JNK) pathways[53,54]. ASK1 is a carrier protein that has been shown to carry mHtt analogue to the nucleus, promoting aggregate formations[55]. Another regulator of autophagy, rapamycin has also been reported to decrease mHtt aggregates and improve neuronal survival in *Drosophila* HD models[56].

The essential autophagy regulator, Beclin-1, exhibits inclusion-like distribution in HD patient brain samples, co-localizing with Htt and blocking macro-autophagy signal transduction processes, enhancing ER stress[57]. Additionally, the sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA), has also been found to significantly contribute to the etiology of HD through the luminal Ca^{2+}-binding proteins (CaBPs) such as calnexin and calreticulin which play an important role in regulating the activity of the SERCA pump[58]. Htt plays a direct role in Ca^{2+} signalling by modulating InsP3R (inositol triphosphate receptor) sensitivity to InsP3 (inositol triphosphate). The InsP3R1–HAP1A-Htt ternary complex has been identified in yeast two-hybrid systems, which sensitizes Insp3R1 to Insp3, and negatively modulates cellular calcium levels[59,60]. Overexpression of the SOC (store-operated calcium channels) pathway in HD neurons increases cellular free calcium ions. A study conducted on transgenic *Drosophila* flies identified the TRPC-1 (transient receptor potential canonical-1) channels associated with the SOC pathway as a novel therapeutic target that mitigates the toxic effects of such pathways[61]. It is known that the synaptic neuronal SOC pathway is controlled by the ER-resident protein STIM2 (stromal interaction molecule-2). A study has revealed that elevated levels of STIM2 in transgenic mice models progresses with age-dependent dendritic spine loss in MSNs[62].

Neuronal excitotoxicity and disrupted axonal transport

Excitotoxicity refers to neuronal cell death caused by the activation of excitatory amino acid receptors[63]. The N-methyl-D-aspartate subclass of ionotropic glutamate receptors (NMDARs) is found to be the most selective and effective in mediating this damage. It has been previously reported that neurons expressing high levels of NMDARs are lost early from the striatum of individuals affected with HD, and injection of NMDAR agonists into the striatum of rodents or non-human primates recapitulates the pattern of neuronal damage observed in HD[64]. Overstimulation of these receptors or an increase in the release/decrease in the uptake of glutamate is known as 'excitotoxicity' and is linked to the pathogenesis of HD. Overexcitation due to the presence of excess glutamates induces depolarization of neuron membranes, followed by activation of ionotropic receptors. Neurons in this state face a rapid influx of water and calcium ions, hastening degeneration[65].

In HD increased glutamate release occurs at early stages, followed by the loss of glutamatergic terminals in fully symptomatic HD, indicating disconnection of the cortex and striatum[66]. Initially elevated glutamate signalling could be a result of higher uptake of extracellular glutamate. At corticostriatal synapses, glutamate is removed from the extracellular space by astrocytes that express glutamate transporters (GLT). Reduced GLT1 mRNA and a corresponding decrease in glutamate have been described in rodent models of HD[67,68]. Overactivation of NMDARs can render neurons susceptible to excitotoxicity, confirmed with the injection of a selective NMDAR agonist quinolinic acid in rodents, which mimicked HD loss of striatal MSNs[69]. Stratial output is governed by the direct and indirect pathways of the MSNs that are differentially vulnerable as the disease progresses. They are composed of dopaminergic (DA) neurons expressing D1 receptors and D2 receptors respectively[70]. Cepeda *et al.*[71] showed that the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) as well as the amplitude of excitotoxic and excitatory effects were higher in indirect compared to direct pathway MSNs. YAC128 (yeast artificial chromosome 128) mice and BACHD (bacterial artificial chromosome) are models expressing 128 and 97 repeats respectively, commonly used in the study of HD[72]. Administering tetrabenazine helped restore dopamine modulation of sEPCs in YAC128 mice and in BACHD

mice rescued eletrophysiological alterations and increased motor function[70,72].

Axonal transport delivers proteins, lipids, mRNA and mitochondria from the cyton to the distal synapse, and clears recycled or misfolded proteins. It is responsible for functions such as neurotransmission, neural trophic signalling and stress insult responses[73]. Motor proteins such as dynein and dynactin help transport proteins across the length of the axon[73,74]. In neurons adversely affected in HD patients, axonal transport is impaired mostly due to interactions of mutant protein with axonal cargo. These abnormal interactions block organelle and protein transport along the axons, ultimately leading to synaptic starvation. Reddy *et al.*[75] used live-cell imaging tools to compare axonal transport in neurons of BACHD and wild-type mice. They reported a significant decrease in mitochondrial motility, especially in anti-retrograde transport across primary neurons in BACHD mice[75]. Another group aiming to understand defects in axonal transport showed that in the Hdh150Q mice model, BDNF transport is disrupted in striatal and not cortical neurons[76]. Lee *et al.*[77] observed in *Drosophila* models that expression of Htt-128 was more diffused and in a non-aggregated state in muscle and epidermal cells, suggesting that some tissues may be more resistant to Htt aggregation than others.

In summary, the mutant protein takes on a toxic gain of function. Its ability to evade, resist and interfere with the other proteins and molecular mechanisms damages the neurons over time. Figure 1 shows the key players and processes occurring in an HD-affected neuron. The figure highlights that the combinatorial effect of failing systems produces an outcome worse than its individual parts.

The intracellular dynamics have been revealed from mHtt interactions with several other nuclear and cytoplasmic entities disrupting cellular homeostasis. The red crosses in Figure 1 indicate a decrease in the number of proteins over the course of the disease. The figure gives a detailed key illustrating the proteins and molecules corresponding to each symbol.

Therapeutic approaches for treating HD

The mutant protein causes neurodegeneration through several cellular mechanisms. Studies using different model systems have helped identify druggable pathways and molecular targets. Treatments ranging from gene therapy strategies to conventional pharmaceutical approaches are currently available for managing HD.

Gene therapy strategies

Novel therapeutic strategies are proving to be powerful and specific methods for gene silencing or Htt inactivation. In HD, an *in vivo* study found four potential lead ASOs (antisense oligonucleotide therapies) specific to mutant protein based on the principle that CAG tract expansion is associated with single nucleotide polymorphisms (SNPs) that can be targeted[78]. The use of interfering RNAs encoded by viral vectors enables RNase H-mediated cleavage of complementary mHtt RNA[78,79]. The use of siRNAs (small interfering RNAs) expressed from miRNA (microRNA) scaffolds driven by the polymerase (pol) III or pol II promoters, allowing for tissue- or cell-specific expression of interfering RNAs increases treatment specificity. High stability of these constructs ensures lower dosage frequency, higher patient compliance and increased safety when the dose administered is maintained within the therapeutic index[80,81]. The use of CRISPR-CAs9 constructs produces precise knicks capable of disrupting translation or inactivating mutant proteins due to frameshift mutations[82]. Another strategy still in its nascent stages of development, aims at improving neuronal homeostasis using selenium nanoparticles and has been shown to work in HD *Caenorhabditis elegans* models[83]. Recently, cell transplantation has gained clinical attention, especially after multicentric intracerebral grafting (MIG-HD), a randomized phase-II trial was conducted. Despite its limited benefits, it has been purported to pave the way to complementary therapy that can reverse tissue atrophy and improve neuronal health[84].

Apart from the therapeutic aspect, such technology can help us realize possible solutions to important questions related to HD pathobiology, which in turn may provide targeted solutions. This symbiosis between technology and therapy may quickly advance these unconventional strategies, thus helping to cure rather than simply delay the disease.

Pharmaceutical and biopharmaceutical strategies

A more traditional approach to the management of HD is through prescribed drugs that provide significant symptomatic relief; however, they fail to reverse the disease conditions. It should be noted that drugs alone have not been found to completely alleviate symptoms as of now. There are reports that point out the use of curcumin, in ameliorating disease symptoms in the *Drosophila* model of HD by suppressing cell death[85].

An interesting study with ciliary neurotrophic factor (CNTF) has shown that is protects striatal neurons in various disease models. Results obtained mainly indicate that to achieve consistent electrophysiological relief, there is a need to reduce biological heterogeneity by modifying the capsule delivery system before conducting further trials[86]. Another important drug candidate, viz. HDAC inhibitor phenylbutyrate is in phase-II clinical trials for HD, and studies have shown promising results[87]. In patients with mild symptoms, a therapy consisting of antioxidants in conjugation with creatine is administered. Creatine plays a major role in intracellular energy buffering in conjunction with phosphocreatine and is effective against superoxide, peroxynitrite

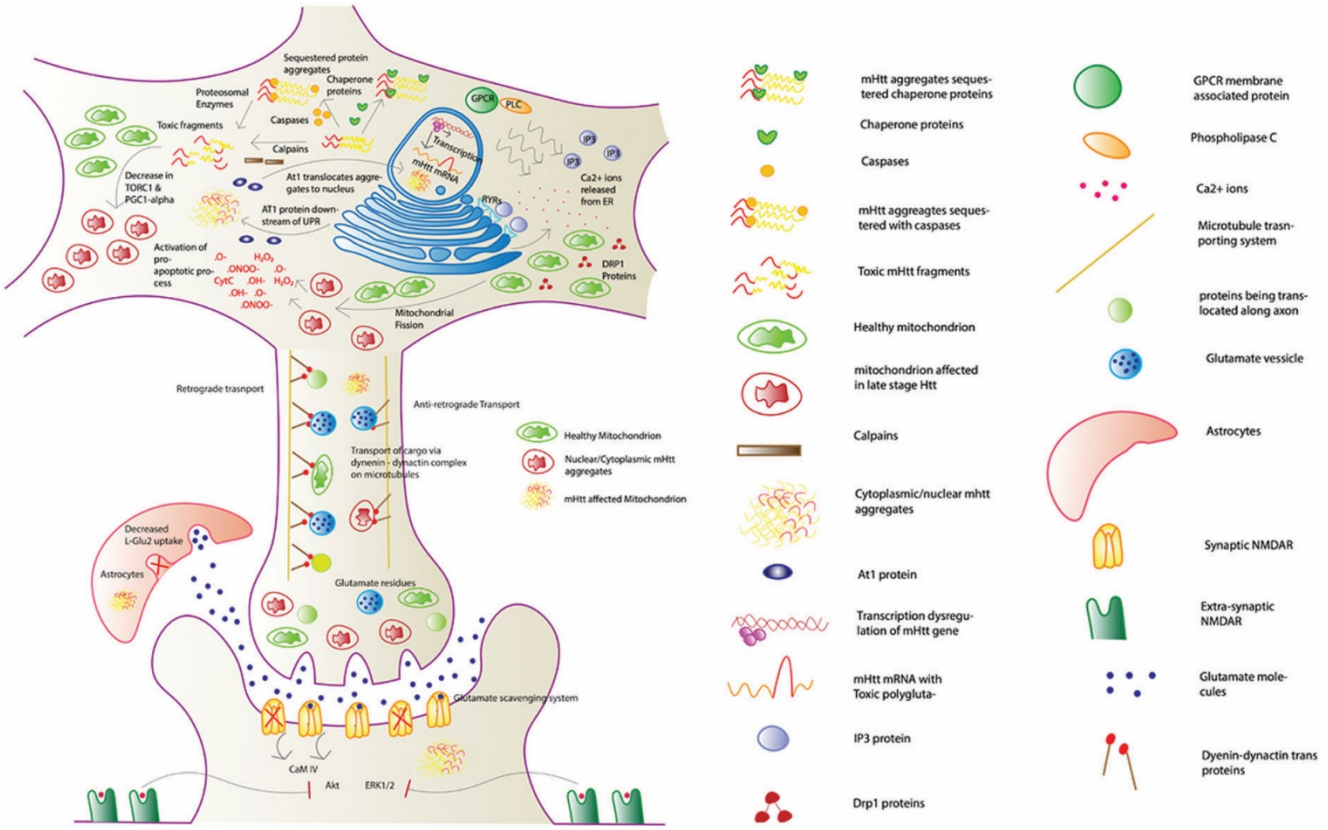

Figure 1. The cellular landscape of a neuron affected by Huntington's disease.

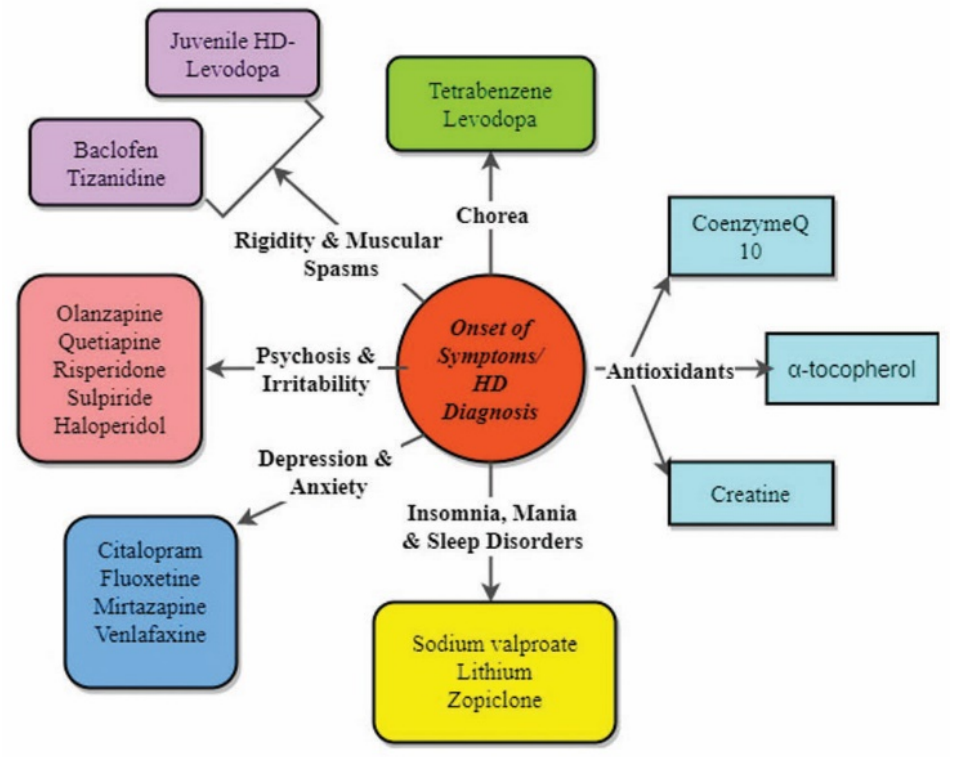

Figure 2. Classically prescribed drug agents against their respective symptoms in HD. Preferred drug classes as well as examples of newer available anti-oxidant-based therapy are represented here.

and hydroxyl radicals[88]. α-Tocopherol (vitamin E) is a lipid-soluble antioxidant that protects cell membranes from oxidation and acts via the glutathione peroxidase pathway. The role of CoenzymeQ10 (CoQ10) is to prevent the oxidation of bases in the mitochondria, and the formation of lipid peroxyl radicals and protein oxidation in lipid membranes[89]. Figure 2 describes various drugs administered according to symptoms experienced by HD patients.

The figure shows classically prescribed drug agents against their respective symptoms in HD. It highlights preferred drug classes and provides examples of new antioxidant-based therapies.

Inter-patient differences commonly occur in genetics, disease onset, variety and severity of symptoms and drug-induced side effects. Thus, it is advised that patients be supplemented with non-pharmacological therapy along with their conventional drug regimen. This is mediated by specialists, including but not limited to a genetic counsellor, a mental health professional, a physical therapist, and a social worker for support and coordination of services[90].

Conclusion

Huntington's chorea is an autosomal dominant disorder characterized by its ability to socially, mentally and physically impair patients in their adult life. However, with the advancement and use of sophisticated modern molecular techniques, we have a good understanding of the several mechanisms involved in its pathogenesis. With the plethora of data currently available and the increasing impetus over the last decade for employing multidisciplinary approaches for disease elucidation, professionals can consider using machine learning and neural networks to simulate the neurodegenerative interactions occurring in the brain. Since HD is a cohesive amalgamation of several toxic cascades that show high variability in frequency and severity among patients, researchers may consider simulation-based approaches to analyse and fill in the gaps of knowledge that currently exist regarding this disease.

Conflict of interest: The authors declare that there is no conflict of interest.

1. Walker, F. O., Huntington's disease. *Lancet*, 2007, **369**(9557), 218–228.
2. Kirkwood, S. C., Su, J. L., Conneally, P. M. and Foroud, T., Progression of symptoms in the early and middle stages of Huntington disease. *Arch. Neurol.*, 2001, **58**(2), 273–278.
3. Feigin, A. S. and Anderson, K. E. (eds), *SPEC – Handbook of Clinical Neurology*, Elsevier, Amsterdam, 2017, vol. 144, pp. 47–48.
4. Vonsattel, J. P., Myers, R. H., Stevens, T. J., Ferrante, R. J., Paskevich, P. A., Richardson, E. P. and Bird, E. D., Huntington's disease: neuropathological grading. In *The Basal Ganglia II*, Springer, Boston, MA, USA, 1987, pp. 515–531.
5. Gutekunst, C. A., Norflus, F. and Hersch, S. M., The neuropathology of Huntington's disease. *Oxf. Monogr. Med. Genet.*, 2002, **45**(1), 251–275.
6. MacDonald, M. E. *et al.*, A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell*, 1993, **72**(6), 971–983.
7. Yanai, A. *et al.*, Palmitoylation of huntingtin by HIP14 is essential for its trafficking and function. *Nature Neurosci.*, 2006, **9**(6), 824–831.
8. Bates, G. P. *et al.*, Huntington disease. *Nature Rev. Dis. Primer.*, 2015, **1**(1), 1–21.
9. Guo, Q. *et al.*, The cryo-electron microscopy structure of huntingtin. *Nature*, 2018, **555**(7694), 117–120.
10. De Rooij, K. E. *et al.*, Dynamic mutation in Dutch Huntington's disease patients: increased paternal repeat instability extending to within the normal size range. *J. Med. Genet.*, 1993, **30**(12), 996–1002.
11. Snell, R. G. *et al.*, Relationship between trinucleotide repeat expansion and phenotypic variation in Huntington's disease. *Nature Genet.*, 1993, **4**(4), 393–397.
12. Landles, C. and Bates, G. P., Huntingtin and the molecular pathogenesis of Huntington's disease: fourth in Molecular Medicine Review Series. *EMBO Rep.*, 2004, **5**(10), 958–963.
13. Imarisio, S. *et al.*, Huntington's disease: from pathology and genetics to potential therapies. *Biochem. J.*, 2008, **412**(2), 191–209.
14. Schulte, J. and Littleton, J. T., The biological function of the Huntingtin protein and its relevance to Huntington's disease pathology. *Curr. Trends Neurol.*, 2011, **5**, 65.
15. Ross, C. A., Huntington's disease: new paths to pathogenesis. *Cell*, 2004, **118**(1), 4–7.
16. Ross, C. A. and Tabrizi, S. J., Huntington's disease: from molecular pathogenesis to clinical treatment. *Lancet Neurol.*, 2011, **10**(1), 83–98.
17. Zielonka, D. *et al.*, The influence of gender on phenotype and disease progression in patients with Huntington's disease. *Parkinson. Rel. Disord.*, 2013, **19**(2), 192–197.
18. Kearney, H. M., Thorland, E. C., Brown, K. K., Quintero-Rivera, F. and South, S. T., American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. *Genet. Med.*, 2011, **13**(7), 680–685.
19. Stout, J. C. *et al.*, Neurocognitive signs in prodromal Huntington disease. *Neuropsychology*, 2011, **25**(1), 1.
20. Paulsen, J. S., Cognitive impairment in Huntington disease: diagnosis and treatment. *Curr. Neurol. Neurosci. Rep.*, 2011, **11**(5), 474–483.
21. Paulsen, J. S. *et al.*, Preparing for preventive clinical trials: the predict-HD study. *Arch. Neurol.*, 2006, **63**(6), 883–890.
22. Klöppel, S. *et al.* and PREDICT-HD investigators of the Huntington Study Group, Automatic detection of preclinical neurodegeneration: presymptomatic Huntington disease. *Neurology*, 2009, **72**(5), 426–431.
23. Ross, C. A. *et al.*, Huntington disease: natural history, biomarkers and prospects for therapeutics. *Nature Rev. Neurol.*, 2014, **10**(4), 204–216.
24. Ciechanover, A., The ubiquitin–proteasome pathway: on protein death and cell life. *EMBO J.*, 1998, **17**(24), 7151–7160.
25. Díaz-Hernández, M. *et al.*, Inhibition of 26S proteasome activity by huntingtin filaments but not inclusion bodies isolated from mouse and human brain. *J. Neurochem.*, 2006, **98**(5), 1585–1596.
26. Jana, N. R., Zemskov, E. A., Wang, G. H. and Nukina, N., Altered proteasomal function due to the expression of polyglutamine-expanded truncated N-terminal huntingtin induces apoptosis by caspase activation through mitochondrial cytochrome c release. *Hum. Mol. Genet.*, 2001, **10**(10), 1049–1059.
27. Chan, H. E., Warrick, J. M., Gray-Board, G. L., Paulson, H. L. and Bonini, N. M., Mechanisms of chaperone suppression of polyglutamine disease: selectivity, synergy and modulation of protein solubility in *Drosophila*. *Hum. Mol. Genet.*, 2000, **9**(19), 2811–2820.
28. Hay, D. G. *et al.*, Progressive decrease in chaperone protein levels in a mouse model of Huntington's disease and induction of stress proteins as a therapeutic approach. *Hum. Mol. Genet.*, 2004, **13**(13), 1389–1405.

29. Goldberg, Y. P. *et al.*, Cleavage of huntingtin by apopain, a proapoptotic cysteine protease, is modulated by the polyglutamine tract. *Nature Genet.*, 1996, **13**(4), 442–449.
30. Wellington, C. L. *et al.*, Inhibiting caspase cleavage of huntingtin reduces toxicity and aggregate formation in neuronal and non-neuronal cells. *J. Biol. Chem.*, 2000, **275**(26), 19831–19838.
31. Ona, V. O. *et al.*, Inhibition of caspase-1 slows disease progression in a mouse model of Huntington's disease. *Nature*, 1999, **399**(6733), 263–267.
32. 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.*, 2000, **6**(7), 797–801.
33. Blum, D., Galas, M. C., Gall, D., Cuvelier, L. and Schiffmann, S. N., Striatal and cortical neurochemical changes induced by chronic neurochemical changes induced by chronic metabolic compromise in the 3-nitropropionic model of Hungtington's disease. *Neurobiol. Dis*., 2002, **10**(3), 410–426.
34. Kim, M., Roh, J. K., Yoon, B. W., Kang, L., Kim, Y. J., Aronin, N. and DiFiglia, M., Huntingtin is degraded to small fragments by calpain after ischemic injury. *Exp. Neurol.*, 2003, **183**(1), 109–115.
35. Gafni, J., Hermel, E., Young, J. E., Wellington, C. L., Hayden, M. R. and Ellerby, L. M., Inhibition of calpain cleavage of huntingtin reduces toxicity: accumulation of calpain/caspase fragments in the nucleus. *J. Biol. Chem.*, 2004, **279**(19), 20211–20220.
36. Cherubini, M., Lopez-Molina, L. and Gines, S., Mitochondrial fission in Huntington's disease mouse striatum disrupts ER-mitochondria contacts leading to disturbances in Ca^{2+} efflux and reactive oxygen species (ROS) homeostasis. *Neurobiol. Dis.*, 2020, **136**, 104741.
37. Giacomello, M., Hudec, R. and Lopreiato, R., Huntington's disease, calcium, and mitochondria. *Biofactors*, 2011, **37**(3), 206–218.
38. Reddy, P. H., Increased mitochondrial fission and neuronal dysfunction in Huntington's disease: implications for molecular inhibitors of excessive mitochondrial fission. *Drug Discov. Today*, 2014, **19**(7), 951–955.
39. Brown, G. C., Murphy, M. P., Scott, I. and Youle, R. J., Mitochondrial fission and fusion. *Essays Biochem.*, 2010, **47**, 85–98.
40. Shirendeb, U., Reddy, A. P., Manczak, M., Calkins, M. J., Mao, P., Tagle, D. A. and Hemachandra Reddy, P., Abnormal mitochondrial dynamics, mitochondrial loss and mutant huntingtin oligomers in Huntington's disease: implications for selective neuronal damage. *Hum. Mol. Genet.*, 2011, **20**(7), 1438–1455.
41. Song, W. *et al.*, Mutant huntingtin binds the mitochondrial fission GTPase dynamin-related protein-1 and increases its enzymatic activity. *Nature Med.*, 2011, **17**(3), 377–382.
42. Shirendeb, U. P. *et al.*, Mutant huntingtin's interaction with mitochondrial protein Drp1 impairs mitochondrial biogenesis and causes defective axonal transport and synaptic degeneration in Huntington's disease. *Hum. Mol. Genet.*, 2012, **21**(2), 406–420.
43. Wu, Z. *et al.*, Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell*, 1999, **98**(1), 115–124.
44. Milakovic, T., Quintanilla, R. A. and Johnson, G. V., Mutant huntingtin expression induces mitochondrial calcium handling defects in clonal striatal cells. *J. Biol. Chem.*, 2006, **281**(46), 34785–34795.
45. Xiang, Z. *et al.*, Peroxisome-proliferator-activated receptor gamma coactivator 1α contributes to dysmyelination in experimental models of Huntington's disease. *J. Neurosci.*, 2011, **31**(26), 9544–9553.
46. St-Pierre, J. *et al.*, Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell*, 2006, **127**(2), 397–408.
47. Reddy, P. H., Mao, P. and Manczak, M., Mitochondrial structural and functional dynamics in Huntington's disease. *Brain Res. Rev.*, 2009, **61**(1), 33–48.
48. Valencia, A. *et al.*, Elevated NADPH oxidase activity contributes to oxidative stress and cell death in Huntington's disease. *Hum. Mol. Genet.*, 2013, **22**(6), 1112.
49. Choo, Y. S., Johnson, G. V., MacDonald, M., Detloff, P. J. and Lesort, M., Mutant Huntingtin directly increases susceptibility of mitochondria to the calcium-induced permeability transition and cytochrome *c* release. *Hum. Mol. Genet.*, 2004, **13**(14), 1407–1420.
50. Shacham, T., Sharma, N. and Lederkremer, G. Z., Protein misfolding and ER stress in Huntington's disease. *Front. Mol. Biosci.*, 2019, **6**, 20.
51. Vembar, S. S. and Brodsky, J. L., One step at a time: endoplasmic reticulum-associated degradation. *Nature Rev. Mol. Cell Biol.*, 2008, **9**(12), 944–957.
52. Yang, H., Liu, C., Zhong, Y., Luo, S., Monteiro, M. J. and Fang, S., Huntingtin interacts with the cue domain of gp78 and inhibits gp78 binding to ubiquitin and p97/VCP. *PLoS ONE*, 2010, **5**(1), e8905.
53. Urano, F., Wang, X., Bertolotti, A., Zhang, Y., Chung, P., Harding, H. P. and Ron, D., Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science*, 2000, **287**(5453), 664–666.
54. Kim, I. *et al.*, Chemical biology investigation of cell death pathways activated by endoplasmic reticulum stress reveals cytoprotective modulators of ASK1. *J. Biol. Chem.*, 2009, **284**(3), 1593–1603.
55. Nishitoh, H. *et al.*, ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats. *Genes Dev.*, 2002, **16**(11), 1345–1355.
56. Ravikumar, B. *et al.*, Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. *Nature Genet.*, 2004, **36**(6), 585–595.
57. Shibata, M. *et al.*, Regulation of intracellular accumulation of mutant Huntingtin by Beclin 1. *J. Biol. Chem.*, 2006, **281**(20), 14474–14485.
58. Roderick, H. L., Lechleiter, J. D. and Camacho, P., Cytosolic phosphorylation of calnexin controls intracellular Ca^{2+} oscillations via an interaction with SERCA2b. *J. Cell Biol.*, 2000, **149**(6), 1235–1248.
59. Choo, Y. S., Johnson, G. V., MacDonald, M., Detloff, P. J. and Lesort, M., Mutant huntingtin directly increases susceptibility of mitochondria to the calcium-induced permeability transition and cytochrome *c* release. *Hum. Mol. Genet.*, 2004, **13**(14), 1407–1420.
60. Milakovic, T., Quintanilla, R. A. and Johnson, G. V., Mutant huntingtin expression induces mitochondrial calcium handling defects in clonal striatal cells. *J. Biol. Chem.*, 2006, **281**(46), 34785–34795.
61. Wu, J. *et al.*, Neuronal store-operated calcium entry pathway as a novel therapeutic target for Huntington's disease treatment. *Chem. Biol.*, 2011, **18**(6), 777–793.
62. Wu, J., Ryskamp, D. A., Liang, X., Egorova, P., Zakharova, O., Hung, G. and Bezprozvanny, I., Enhanced store-operated calcium entry leads to striatal synaptic loss in a Huntington's disease mouse model. *J. Neurosci.*, 2016, **36**(1), 125–141.
63. Beal, M. F., Mechanisms of excitotoxicity in neurologic diseases. *FASEB J.*, 1992, **6**(15), 3338–3344.
64. Fan, M. M. and Raymond, L. A., *N*-methyl-D-aspartate (NMDA) receptor function and excitotoxicity in Huntington's disease. *Prog. Neurobiol.*, 2007, **81**(5–6), 272–293.
65. Raymond, L. A., Excitotoxicity in Huntington disease. *Clin. Neurosci. Res.*, 2003, **3**(3), 121–128.
66. Sepers, M. D. and Raymond, L. A., Mechanisms of synaptic dysfunction and excitotoxicity in Huntington's disease. *Drug Discov. Today*, 2014, **19**(7), 990–996.
67. Miller, B. R., Dorner, J. L., Bunner, K. D., Gaither, T. W., Klein, E. L., Barton, S. J. and Rebec, G. V., Up-regulation of GLT1 reverses the deficit in cortically evoked striatal ascorbate efflux in the R6/2 mouse model of Huntington's disease. *J. Neurochem.*, 2012, **121**(4), 629–638.
68. Estrada-Sánchez, A. M. and Rebec, G. V., Corticostriatal dysfunction and glutamate transporter 1 (GLT1) in Huntington's disease: interactions between neurons and astrocytes. *Basal Ganglia*, 2012, **2**(2), 57–66.
69. Beal, M. F., Kowall, N. W., Ellison, D. W., Mazurek, M. F., Swartz, K. J. and Martin, J. B., Replication of the neurochemical

characteristics of Huntington's disease by quinolinic acid. *Nature*, 1986, **321**(6066), 168–171.
70. Galvan, L., André, V. M., Wang, E. A., Cepeda, C. and Levine, M. S., Functional differences between direct and indirect striatal output pathways in Huntington's disease. *J. Huntington's Dis.*, 2012, **1**(1), 17–25.
71. Cepeda, C., André, V. M., Yamazaki, I., Wu, N., Kleiman-Weiner, M. and Levine, M. S., Differential electrophysiological properties of dopamine D1 and D2 receptor-containing striatal medium-sized spiny neurons. *Eur. J. Neurosci.*, 2008, **27**(3), 671–682.
72. André, V. M., Fisher, Y. E. and Levine, M. S., Altered balance of activity in the striatal direct and indirect pathways in mouse models of Huntington's disease. *Front. Syst. Neurosci.*, 2011, **5**, 46.
73. Millecamps, S. and Julien, J. P., Axonal transport deficits and neurodegenerative diseases. *Nature Rev. Neurosci.*, 2013, **14**(3), 161–176.
74. Perlson, E., Maday, S., Fu, M. M., Moughamian, A. J. and Holzbaur, E. L., Retrograde axonal transport: pathways to cell death? *Trends Neurosci.*, 2010, **33**(7), 335–344.
75. Reddy, P. H. and Shirendeb, U. P., Mutant huntingtin, abnormal mitochondrial dynamics, defective axonal transport of mitochondria, and selective synaptic degeneration in Huntington's disease. *Biochim. Biophys. Acta – Mol. Basis Dis.*, 2012, **1822**(2), 101–110.
76. Her, L. S. and Goldstein, L. S., Enhanced sensitivity of striatal neurons to axonal transport defects induced by mutant huntingtin. *J. Neurosci.*, 2008, **28**(50), 13662–13672.
77. Lee, W. C. M., Yoshihara, M. and Littleton, J. T., Cytoplasmic aggregates trap polyglutamine-containing proteins and block axonal transport in a *Drosophila* model of Huntington's disease. *Proc. Natl. Acad. Sci. USA*, 2004, **101**(9), 3224–3229.
78. Lima, W. F. *et al.*, Single-stranded siRNAs activate RNAi in animals. *Cell*, 2012, **150**(5), 883–894.
79. Kay, C., Skotte, N. H., Southwell, A. L. and Hayden, M. R., Personalized gene silencing therapeutics for Huntington disease. *Clin. Genet.*, 2014, **86**(1), 29–36.
80. Stanek, L. M. *et al.*, Silencing mutant huntingtin by adeno-associated virus-mediated RNA interference ameliorates disease manifestations in the YAC128 mouse model of Huntington's disease. *Hum. Gene Ther.*, 2014, **25**(5), 461–474.
81. Southwell, A. L., Skotte, N. H., Bennett, C. F. and Hayden, M. R., Antisense oligonucleotide therapeutics for inherited neurodegenerative diseases. *Trends Mol. Med.*, 2012, **18**(11), 634–643.
82. Dabrowska, M. and Olejniczak, M., Gene therapy for Huntington's disease using targeted endonucleases. In *Trinucleotide Repeats*, Humana, New York, NY, USA, 2020, pp. 269–284.
83. Cong, W., Bai, R., Li, Y. F., Wang, L. and Chen, C., Selenium nanoparticles as an efficient nanomedicine for the therapy of Huntington's disease. *ACS Appl. Mater. Interfaces*, 2019, **11**(38), 34725–34735.
84. Bachoud-Lévi, A. C. *et al.*, Human fetal cell therapy in Huntington's disease: a randomized, multicenter, phase II trial. *Mov. Disord.*, 2020, **35**(8), 1323–1335.
85. Chongtham, A. and Agrawal, N., Curcumin modulates cell death and is protective in Huntington's disease model. *Sci. Rep.*, 2016, **6**(1), 1–10.
86. Bachoud-Lévi, A. C. *et al.*, Neuroprotective gene therapy for Huntington's disease using a polymer encapsulated BHK cell line engineered to secrete human CNTF. *Hum. Gene Ther.*, 2000, **11**(12), 1723–1729.
87. Hogarth, P., Lovrecic, L. and Krainc, D., Sodium phenylbutyrate in Huntington's disease: a dose-finding study. *Mov. Disord.*, 2007, **22**(13), 1962–1964.
88. Tabrizi, S. J., Blamire, A. M., Manners, D. N., Rajagopalan, B., Styles, P., Schapira, A. H. V. and Warner, T. T., Creatine therapy for Huntington's disease: clinical and MRS findings in a 1-year pilot study. *Neurology*, 2003, **61**(1), 141–142.
89. Johri, A. and Beal, M. F., Antioxidants in Huntington's disease. *Biochim. Biophys. Acta – Mol. Basis Dis.*, 2012, **1822**(5), 664–674.
90. Adam, O. R. and Jankovic, J., Symptomatic treatment of Huntington disease. *Neurotherapeutics*, 2008, **5**(2), 181–197.

ACKNOWLEDGEMENTS. We thank Siddhant Narula and Eashan Chopde for help with the illustrations. We also thank members of the Tare laboratory at BITS Pilani for providing valuable inputs.

Received 1 August 2022; revised accepted 23 February 2023

doi: 10.18520/cs/v124/i10/1151-1159