

Huntington's disease: a clinical review

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Abstract

Huntington's disease (HD) is a fully penetrant neurodegenerative disease caused by a dominantly inherited CAG trinucleotide repeat expansion in the huntingtin gene on chromosome 4. In Western populations HD has a prevalence of 10.6-13.7 individuals per 100,000. It is characterised by cognitive, motor and psychiatric disturbance. At the cellular level mutant huntingtin results in neuronal dysfunction and death through a number of mechanisms, including disruption of proteostasis, transcription and mitochondrial function and direct toxicity of the mutant protein. Early macroscopic changes are seen in the striatum with involvement of the cortex as the disease progresses. There are currently no disease modifying treatments therefore supportive and symptomatic management is the mainstay of treatment. In recent years there have been significant advances in understanding both the cellular pathology and the macroscopic structural brain changes that occur as the disease progresses. In the last decade there has been a large growth in potential therapeutic targets and clinical trials. Perhaps the most promising of these are the emerging therapies aimed at lowering levels of mutant huntingtin. Antisense oligonucleotide therapy is one such approach with clinical trials currently underway. This may bring us one step closer to treating and potentially preventing this devastating condition.

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Introduction

In 1872 George Huntington wrote an account of hereditary chorea, which we now know as Huntington's disease. He described its hereditary nature, associated psychiatric and cognitive symptoms and the manifestation of the disease in adult life between 30 and 40 years of age. He outlined the progressive nature of the disease stating, "Once it begins it clings to the bitter end" [1]. However the monogenic nature and full penetrance of HD makes it perhaps one of the most treatable neurodegenerative diseases. This has become particularly apparent in the last decade with the advent of new therapeutic approaches that can directly target the HD gene and prevent production of the toxic mutant huntingtin protein [2].

Aetiology

HD is caused by an autosomal dominantly inherited CAG trinucleotide repeat expansion in the huntingtin (*HTT*) Gene on chromosome 4. This results in the production of a mutant huntingtin (mHTT) protein with an abnormally long polyglutamine repeat [3]. Those with greater than 39 CAG repeats are certain to develop the disease, while reduced penetrance is seen between 36 to 39 repeats. Anticipation can be seen when the gene is passed down the paternal line, such that a father with a CAG repeat length in the intermediate range may have a child with an expanded pathogenic repeat length. This is because sperm from males shows greater repeat variability and larger repeat sizes than somatic tissues [4].

Epidemiology

HD has a prevalence of 10.6-13.7 individuals per 100,000 in Western populations. Japan, Taiwan and Hong Kong have a much lower incidence of HD with a prevalence of 1-7 per million, in South Africa lower rates are seen in black populations when compared to white and mixed populations. The difference in disease prevalence across ethnic groups relate to genetic differences in the *HTT* gene. Populations with a high

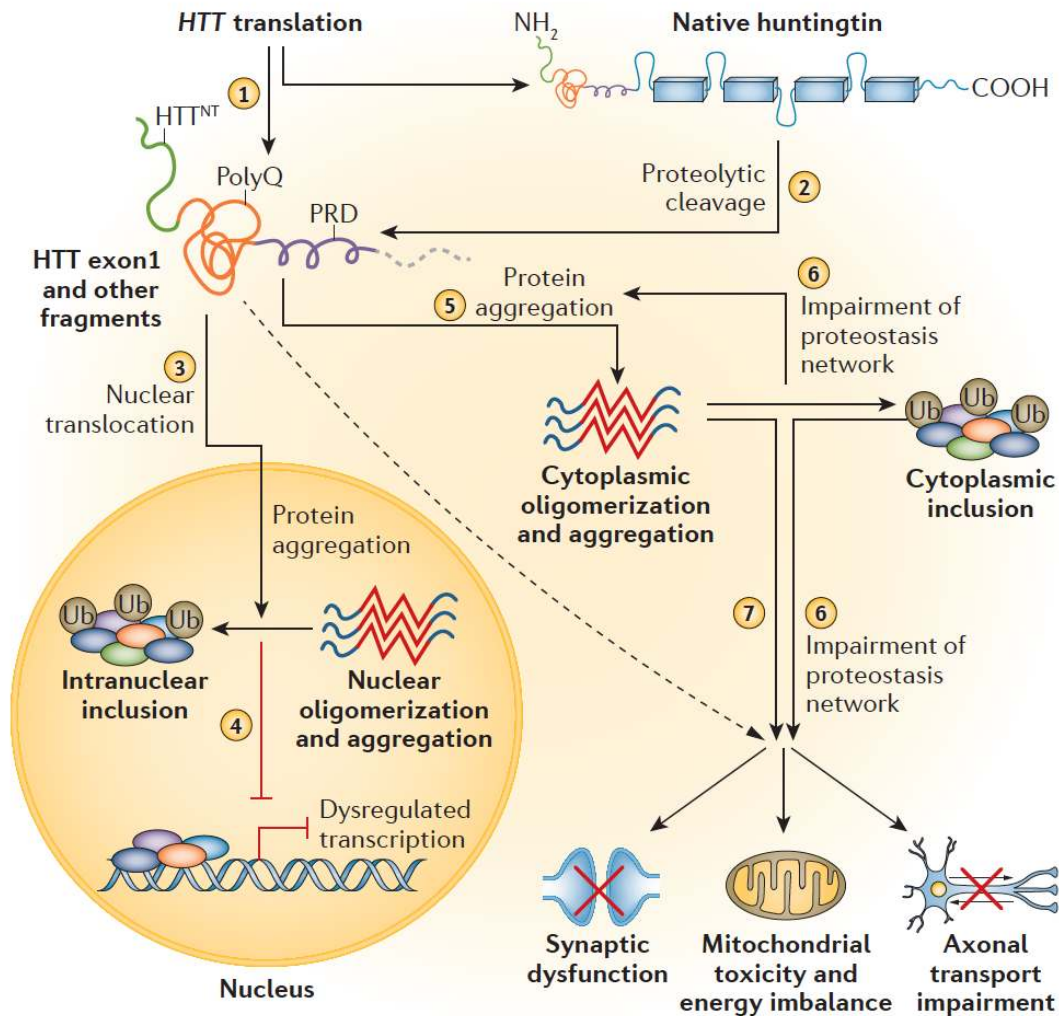
prevalence have longer average CAG repeats. For example those of European ancestry have an average of 18.4-18.7, while those of Asian ancestry have an average of 16.9-17.4 [5].

Pathogenesis

Molecular pathogenesis

mHTT results in neuronal dysfunction and death through a number of mechanisms. These include direct effects from the exon 1 mHTT fragment, the propensity of mHTT to form abnormal aggregates and its effects on cellular proteostasis, axonal transport, transcription, translation, mitochondrial and synaptic function [6, 7] (see figure 1). Medium spiny neurons (MSNs) of the striatum are selectively vulnerable to the effects of mHTT. Striatal pathology follows a biphasic course with initial loss of MSNs of the indirect pathway leading to a hyperkinetic phenotype followed by loss of MSNs of the direct pathway resulting in a hypokinetic phenotype [8]. The cause for the selective vulnerability of indirect pathway MSNs is unclear, however dopamine D2 receptors may be a factor as they are expressed by indirect but not direct MSNs and have been implicated in HD pathogenesis [9], other hypotheses include the loss of brain derived neurotrophic factor, glutamate excitotoxicity from cortico-striatal projections and toxic effects of repeat associated non-ATG (RAN) translation proteins [6][10].

Figure 1. Pathogenetic cellular mechanisms in Huntington disease. (1) *HTT* is translated to produce the full-length huntingtin protein as well as an amino-terminal *HTT* exon1 fragment (the result of aberrant splicing). The length of the polyglutamine (polyQ) tract in these proteins depends on the extent of somatic instability. (2) Full-length native huntingtin is cleaved through proteolysis to generate additional protein fragments. (3) Protein fragments enter the nucleus. (4) Fragments are retained in the nucleus through self-association, oligomerization and aggregation — leading to the formation of inclusions, a process that causes transcriptional dysregulation through the sequestration of other proteins and through other incompletely defined mechanisms. (5) Huntingtin fragments oligomerize and aggregate in the cytoplasm. (6) The aggregation of huntingtin is exacerbated through the disease-related impairment of the proteostasis network, which also leads to global cellular impairments. (7) The aberrant forms of huntingtin result in additional global cellular impairments, including synaptic dysfunction, mitochondrial toxicity and a decreased rate of axonal transport. PRD, proline-rich domain; Ub, ubiquitin. Reproduced with permissions from [7].



Macroscopic pathology

Post-mortem studies reveal diffuse atrophy of the caudate and putamen with degeneration occurring along a caudo-rostral, dorso-ventral and medio-lateral gradient. The globus pallidus and nucleus accumbens are also affected but to a lesser extent [11]. A classification system for HD pathology has been developed which consists of 5 grades. Grade 0: clinical evidence for HD but no gross or microscopic abnormalities that could be related to HD. Grade 1: No macroscopic abnormalities in the caudate or putamen but moderate fibrillary astrocytosis at the microscopic level. Grade 2: Macroscopic changes in the caudate and putamen but no macroscopic changes in the Globus Pallidus. Grade 3: lateral segment of the globus pallidus showing fibrillary astrocytosis with the medial segment of the GP unchanged. Grade 4: Shrunken caudate yellow-brown in colour, widened anterior horn of lateral ventricle and smaller nucleus accumbens [12].

At grades 3 and 4 changes are also seen in other brain regions including the thalamus, sub-thalamic nucleus, white matter and cerebellum. With respect to the cerebral cortex atrophy is variable even in stages 3 and 4 [12]. More recently advances in magnetic resonance imaging (MRI) has confirmed these early pathological findings in vivo, particularly loss caudate and putamen grey matter volume and loss of both striatal and cortical white matter [13].

Clinical features

Diagnosis

Diagnosis of HD is based on a confirmed family history or positive genetic test and the onset of motor disturbance as defined by the Unified HD rating scale (UHDRS) total motor score (TMS) diagnostic confidence score. This score ranges from 0 (no motor abnormalities suggestive of HD) to 4 ($\geq 99\%$ to be due to HD), with a score of 4 defining motor onset or 'manifest' HD. However subtle motor, cognitive and

psychiatric deficits can be identified up to 10-15 before the onset of manifest disease and this is referred to as the premanifest stage of the disease [14].

CAG length and clinical phenotype

The full penetrance of HD in mutation carriers with > 39 CAG repeats makes it an ideal model for studying the preclinical phase of neurodegeneration, as it is possible to predict who will develop the disease many years before symptom onset. Reduced penetrance is seen between 36-39 repeats [15], while 27-35 is considered the intermediate range and below 27 is normal. CAG repeat length accounts for approx. 56% of the variability in age of onset [16] and is also correlated with progression of motor and cognitive deficits [17].

Genetic modifiers

Genetic factors independent of CAG repeat length have also been shown to modify HD. The largest genome wide association study (GWAS) study in HD identified a number of genes involved in DNA repair that can alter the age of motor onset. Two genes on chromosome 15, FAN1 (Fanconi anemia FANCD1/FANCD2-associated endonuclease) and MTMR10 (myotubularin related protein 10) were shown to be the most significant. On chromosome 8 significant associations were also seen with RRM2B (a subunit of DNA damage p53-inducible ribonucleotide reductase M2 B) and URB5 (an HECT domain E3 ubiquitin-protein ligase). Genetic pathway analysis also implicated gene pathways involved in DNA repair, mitochondrial fission and oxidoreductase activity [18]. Similarly a recent GWAS has revealed association between HD progression and a genetic variant in MSH3, a DNA repair gene, associated with CAG somatic instability [19].

Natural history studies

In recent years a number of multi-centre natural history studies have been pivotal in both our understanding of disease onset, progression and in the search for clinical and imaging biomarkers. The largest study to date is Registry, which is a European study spanning 16 countries with over 17,000 participants collecting motor, cognitive, behavioural and biosample data [20]. Co-operative HD Observation Research Trial (COHORT) [21] and Prospective Huntington At Risk Observational Study (PHAROS) [22] are both prospective longitudinal studies tracking changes in motor, cognitive and behavioural variables.

In addition to extensive clinical data PREDICT and Track-HD collected imaging data across multiple time points. PREDICT included over 1,000 participants followed up over 10 years and focused on identifying measures that predict conversion to manifest HD [23]. Track-HD was a study focused on the evaluation of biomarkers for clinical trials and included 123 controls, 120 premanifest and 123 with manifest disease, followed up over 3 years [24]. This has now extended to TrackOn-HD, which aims to identify functional markers of pre-manifest HD and study mechanisms of brain compensation over 3 time points one year apart [25].

Motor disturbance

In keeping with the biphasic course of striatal pathology with initial loss of MSNs of the indirect pathway followed by loss of MSNs of the direct pathway [8], movement disturbance in HD can be split into a hyperkinetic phase with prominent chorea in the early stages of the disease, which then tends to plateau [26]. The hypokinetic phase is characterised by bradykinesia, dystonia, balance and gait disturbance. The hypokinetic movement disorder shows association with disease duration and CAG length while chorea does not [27].

Assessment of motor disturbance is based on the UHDRS TMS, which assesses eye movements, speech, alternating hand movements, dystonia, chorea and gait. While the UHDRS TMS is sensitive to change over time [28] it is also subject to inter-rater variability [29]. More quantitative assessments such as the (quantitative) Q-motor battery, which includes tongue force variability, grip force, speeded and self paced tapping [30], have shown sensitivity to longitudinal change [28].

Cognitive disturbance

Cognitive disturbance can be seen many years before symptom onset and follows a sub-cortical pattern characterized by impaired emotion recognition, processing speed, visuospatial and executive function [31]. In early manifest disease longitudinal changes can be demonstrated over 12 and 24 months by performance on the symbol digit modalities test, which assesses psychomotor speed, Stroop word reading which assesses executive function and indirect circle tracing, which is used to assess visuospatial performance and the emotion recognition test [13, 32]. This extends to premanifest HD at 36-months, with Stroop word reading demonstrating the highest sensitivity for those furthest from disease onset [28].

Neuropsychiatric disturbance

A wide variety of neuropsychiatric symptoms occur in HD, including apathy, anxiety, irritability, depression, obsessive compulsive behaviour and psychosis. While high rates are seen in manifest disease [33], psychiatric disturbance is also common many years before symptom onset in the premanifest stage [24]. The most recent study from the Registry cohort, which includes both premanifest and manifest participants, shows that Apathy is the most common occurring in 28%, while depression, irritability and obsessive compulsive behavior occur in around 13%. Psychosis is relatively rare occurring in 1% [34].

Differential diagnoses

In the absence of a mHTT mutation the triad of chorea, cognitive and neuropsychiatric disturbance is known as a HD phenocopy. While diagnosis can only be achieved in around 3% [36] of these cases there are a number of genetic conditions that may present as HD phenocopies. The most common of these in European populations are C9orf72 [37] and Spinocerebellar ataxia (SCA) 17 [36]. Additional features such as ataxia or peripheral neuropathy may suggest other diagnoses such as SCA 1-3 or Friedrich's ataxia. In the case of seizures Dentatorubral-pallidoluysian atrophy (DRPLA) should be considered. Iron accumulation disorders such as Neuroferritinopathy and Neurodegeneration with brain iron accumulation (NBIA) may reveal abnormal MRI imaging. In the case of Neuroacanthocytosis abnormal acanthocytes can be seen on peripheral blood films. Huntington's disease like syndrome 2 (HDL2) is the most common cause of HD phenocopies in African populations [37, 38].

Isolated chorea can be caused by acquired conditions including striatal pathology, chorea of pregnancy, systemic lupus erythematosus/anti-phospholipid syndrome, thyrotoxicosis, post infectious syndromes, polycythaemia rubra vera and drugs [38].

Investigations

Genetic testing

Genetic testing for the mHTT mutation can either be diagnostic or predictive. In the case of a diagnostic test this may be performed when a patient presents with typical motor features of HD. Prior to testing it is important to inform the patient about HD and its hereditary nature as a positive test has implications both for the patient and their family. Delivering the news of a positive genetic test should be done face-to-face with

the patient and his/her family. The option of referral to a specialist HD management clinic should also be provided [39].

Predictive testing (PT) is done prior to symptom onset in adults who are at risk of inheriting the *HTT* gene mutation. International guidelines were established shortly after the identification of the *HTT* gene in 1993 [3] and have been updated in 2013 [40]. The protocol for predictive testing consists of pretest counseling where the candidate is provided with information in order to make an informed decision regarding the risks and benefits of testing. After a period of time this is followed by a neurological examination to ensure the candidate is not symptomatic and then psychological screening to identify those at high risk of suicide in the event of a positive result. Post-test follow-up is also carried out to monitor the effects of the test result and assess if the candidate requires any further support [41]. Predictive testing is commonly performed for reproductive reasons. Reproductive options for at risk individuals include prenatal diagnosis (PND) and termination of pregnancy in the event that the foetus carries the expanded CAG or pre-implantation genetic diagnosis (PGD) performed during in-vitro fertilisation where only embryos without the CAG expansion are transferred. These options are also available to those unaware of their gene status by use of an exclusion test, which tests for the mutant *HTT* allele of the affected grandparent [42].

Management

The optimal management of HD involves a multidisciplinary approach involving physicians, nurses, physiotherapists, speech and language therapists, dieticians and other health care professionals. The aim is to optimize quality of life and pre-empt the changing needs of the patient as the disease progresses. This usually involves a combination of pharmacological and non-pharmacological interventions. In many instances the evidence base for pharmacological treatments is sparse and decisions are made based on expert opinion and clinical experience [43].

Motor symptoms

Chorea is one of the most prominent symptoms in HD and occurs early in the disease. The only drug specifically licensed to treat chorea is tetrabenazine [44]. This is a synaptic vesicular amine transport inhibitor, which provides a sustained anti-choreic effect at doses in the range 50-75mg per day. Side effects include sleep problems, depression, anxiety and restlessness [45].

Deutetrabenazine is a modified version of Tetrabenazine that contains deuterium molecules. This results in prolonged half-life and less metabolism variability. The FIRST-HD study revealed that compared to placebo Deutetrabenazine significantly reduces chorea [46] and while no head-to-head studies have been performed comparing Tetrabenazine and Deutetrabenazine, there is the suggestion that Deutetrabenazine may result in less side effects, such as depression and somnolence [47].

Sulpiride, a neuroleptic, has shown efficacy in treating chorea in a randomised control trial (RCT). In clinical practice other neuroleptics including olanzapine, risperidone and quetiapine are also commonly used, with sedation and weight gain being the most common side effects [45]. Other motor symptoms such as abnormal gait, poor balance and frequent falls are commonly treated with physiotherapy.

Psychiatric symptoms

There is limited evidence with regard to the treatment of psychiatric symptoms in HD therefore treatment decisions are based on clinical consensus and expert opinion. Depression, anxiety, obsessive compulsive disorder and irritability may be treated with non-pharmacological interventions such as cognitive behavioural therapy or psychodynamic therapy, however these approaches may be limited in the context of cognitive impairment. Pharmacological interventions include selective serotonin uptake inhibitors (citalopram, fluoxetine, paroxetine and sertraline) and Mirtazepine and venlafaxine, which have serotonergic

and noradrenergic effects. Neuroleptics can be useful in treating aggression and psychosis. A number of medications, including methylphenidate, atomoxetine, modafinil, amantadine, bromocriptine and bupropion have been used to treat apathy however no RCTs have been performed [48].

Cognitive symptoms

Two RCTs have assessed the use of anti-cholinesterase inhibitors for cognition in HD, however participant numbers were small and results were conflicting [49]. Another RCT found no effect of citalopram on cognitive function [50]. Coping strategies to deal with cognitive deficits can be beneficial. For example requesting employers change the type or work or work setting, for example working in a quiet environment or changing to work that requires less multi-tasking [51].

Biomarkers

Clinical

The cognitive measures Stroop word reading, symbol digit modalities and circle tracing (direct and indirect) are sensitive to longitudinal change in HD over 24 months, however relatively little change is seen in premanifest HD over this time course [32]. Quantitative measures of chorea, grip force and speeded tapping shows changes in HD over 24 months, with speeded tapping also showing longitudinal change in premanifest HD [52]. Longitudinal change in psychiatric measures is more variable and only seen in apathy [28].

Biofluid Biomarkers

A number of potential blood biomarkers have emerged. The most promising of these is neurofilament light (NFL) protein. Baseline plasma levels of NFL protein show correlation with progression in brain atrophy and motor and cognitive measures and over time. In premanifest HD baseline plasma NFL is associated with disease onset over a 3-year period. Plasma NFL is highly correlated with CSF NFL, suggesting peripheral blood sampling may be sufficient to accurately detect NFL therefore avoiding the need for more invasive CSF collection [53]. Transcription studies using RNA derived from blood reveal abnormal gene expression in HD compared to controls, however results have been conflicting [54, 55]. Increases in immune proteins in plasma, using a proteomics approach, also show correlation with disease stage [56]. More recently it has been possible to detect mHTT from blood-derived monocytes, with levels correlating with disease burden and caudate atrophy [57]. Longitudinal studies are yet to reveal whether these approaches can detect change over time.

Much research has focused on searching for cerebrospinal fluid (CSF) biomarkers [58]. Perhaps the most promising of these is the detection of mHTT in CSF. mHTT in the CSF correlates with disease burden and is also associated with cognitive and motor performance [59]. Other CSF markers are also linked to disease stage, such as tau, neurofilament light and measures of inflammation [60, 61]. HD clarity a large multi-site initiative has recently been set up in order to collect large numbers of CSF samples to facilitate further CSF biomarker investigation (<http://hdclarity.net>).

Imaging

Structural MRI has been the most extensively studied imaging modality in HD to date. Track-HD evaluated changes in both grey and white matter volume at 3 time points one year apart. This study revealed grey

matter volume loss in the striatum and loss of white matter volume around the striatum, within the corpus callosum and in the posterior white matter tracts in the premanifest stage extending to wide spread loss of white matter volume, and to a lesser extent grey matter, in manifest stage [13, 28, 52]. The limited decline in cognitive and motor function in the premanifest stage coupled with this grey and white matter volume has led to the suggestion that compensatory mechanisms such as neuroplasticity and network reconfiguration enable those in the premanifest stage to maintain normal function [52].

Diffusion weighted MRI (DWI) can be used to measure the diffusion of water in-vivo and is therefore capable of providing information about the microstructure of white matter fibre tracts in the brain [62]. In keeping with regional changes in white matter volume, microstructural white matter changes are also seen around the striatum, within the corpus callosum and in the posterior white matter tracts [63]. More recently studies using diffusion tractography, which can delineate white matter connections, have shown selective vulnerability of cortico-striatal white matter connections in premanifest HD, extending to wide spread loss of white matter connections in the manifest stage [64].

Other imaging modalities such as functional MRI have shown abnormalities both in premanifest and manifest disease [65]. Positron emission tomography using a phosphodiesterase 10A tracer has been able to detect change in premanifest HD up to 25 years before symptoms onset even before grey and white matter changes occur [66].

Clinical Trials

Over the past two decades 99 clinical trials have been performed in Huntington's disease investigating 41 different compounds. However success rate is low with only 3.5% of trials progressing to the next stage [67].

Currently there are 23 active clinical trials in HD registered with ClinicalTrials.gov (see table 1). In this section we will review a number of studies that have completed recently.

Pridopidine, a dopamine modulator, has been studied in three large phase 3 trials MermaiHD [68], HART [69] and Pride-HD [70], unfortunately none of these studies reached their primary end points. PBT2 is a metal protein-attenuating compound, which acts to reduce metal induced aggregation of mHTT. In a phase 2 trial the Reach2HD study showed this drug was safe and well tolerated and plans are currently underway for a phase 3 trial [71].

Cysteamine, used in the treatment of cystinosis, increases brain-derived neurotrophic factor, a growth factor depleted in the brains of HD patients [6]. The effect of Cysteamine on motor progression in HD has been evaluated in 3-year long phase 2/3 trial. This revealed that it was safe and well tolerated however effects on motor progression did not reach statistical significance [72]. SIRT1 a member of the Sirtuin family and causes reduction of mHTT protein levels [73]. This molecule has been investigated in a phase 2 study, but no effect on the UHDRS TMS was seen [74].

Phosphodiesterase 10A (PDE10A) is found in the striatum and is reduced in HD patients many years before the onset of manifest disease [66]. Inhibition of this enzyme using PDE10A inhibitors has been shown to restore basal ganglia circuitry in HD animal models [75]. This compound was recently tested in the Pfizer Amaryllis phase 2 trial. Unfortunately this failed to show significant improvement in motor, cognitive or behavioural measures [76].

Table 1. Active clinical trials in Huntington's disease (ClinicalTrials.gov accessed 11th July 2017).

Clinical Trial
Open-label Extension Study of Pridopidine (ACR16) in the Symptomatic Treatment of Huntington Disease Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of IONIS-HTTRx in Patients With Early Manifest Huntington's Disease
Deep Brain Stimulation (DBS) of the Globus Pallidus (GP) in Huntington's Disease (HD)
Resveratrol and Huntington Disease
Exploring Computerised Cognitive Training for People With Huntington's Disease
Exercise Effects in Huntington's Disease
Beta Testing of a New Assessment in Huntington's Disease (HD)
Feasibility of a Video-oculography in Patients With Huntington's Disease VOG-HD Study
A Pilot Evaluation of Mindfulness-based Cognitive Therapy for People With Huntington's Disease
A Comparative Phase 2 Study Assessing the Efficacy of Triheptanoin, an Anaplerotic Therapy in Huntington's Disease
Follow-up Measurement of Brain PDE10A Enzyme Levels in Huntington's Disease Gene Expansion Carriers
A Clinical Study in Subjects With Huntington's Disease to Assess the Efficacy and Safety of Three Oral Doses of Laquinimod
A Study to Evaluate Sigma-1 and Dopamine-2 Receptor Occupancy by Pridopidine in the Human Brain of Healthy Volunteers and in Patients With Huntington's Disease
Tolerability, Safety, and Activity of SRX246 in Irritable Subjects With Huntington's Disease
Effect of Tetrabenazine on Stroop Interference in HD
Social Cognition in Huntington's Disease: Cognitive Study and Functional and Morphological Imaging
Assessment of the Psychological, Cognitive and Social Resources of Applicants for Huntington's Disease and Presymptomatic Genetic Testing
Alternatives for Reducing Chorea in HD
Brain Stimulation in Movement Disorders
Validation and Standardization of a Battery Evaluation of the Socio-emotional Functions in Various Neurological Pathologies (GREFEX II)
Electronic-health Application To Measure Outcomes REMotely Clinical Trial
Laughter Therapy Effects on Mood, Stress and Self-efficacy in People With Neurological Diseases.
ExAblate Transcranial MRgFUS for the Management of Treatment-Refractory Movement Disorders

DNA and RNA targeting therapies for HD

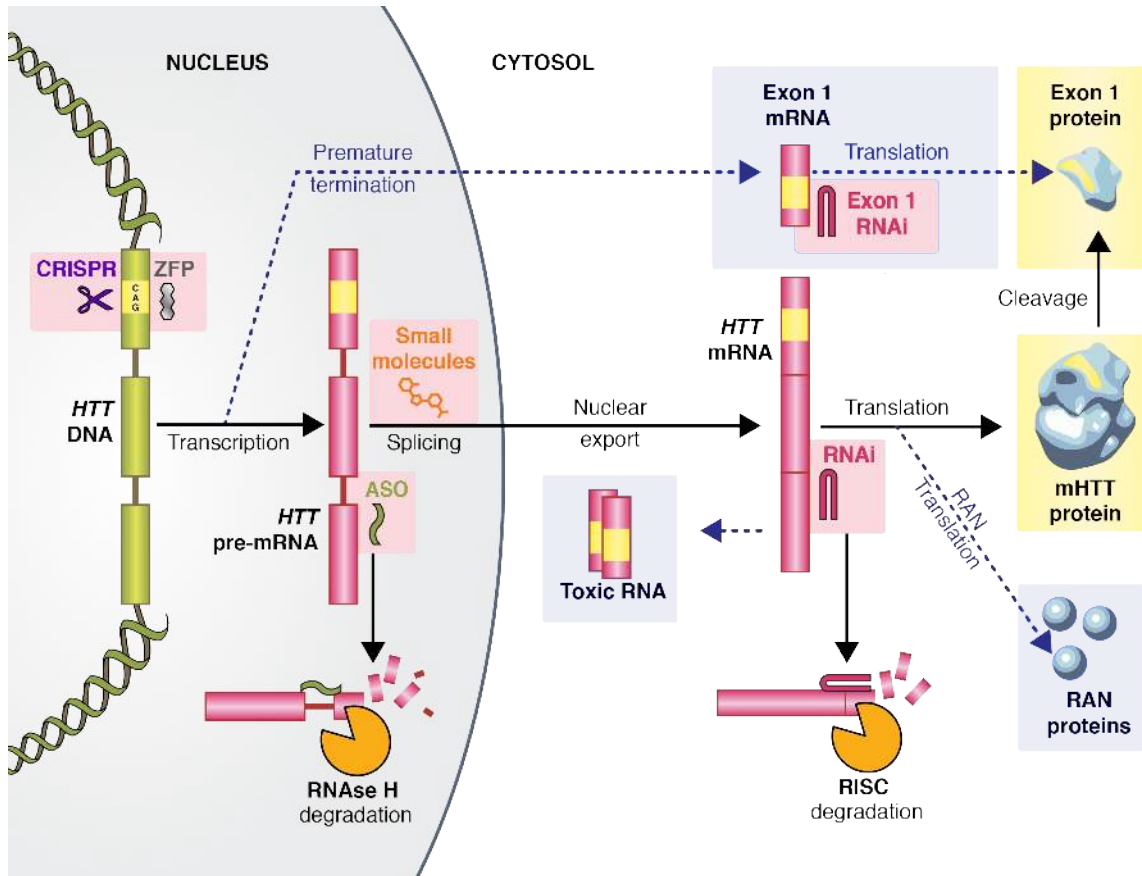
Perhaps the most promising approaches with regards to disease modification are the emerging therapies aimed at lowering levels of mHTT by targeting either the DNA or RNA of the mHTT gene (see figure 3) [2]. RNA targeting can be achieved by using Anti-sense oligonucleotides (ASOs), RNA interference (RNAi) or small molecule splicing inhibitors. ASOs are currently being trialed in a first in human phase 1b/2a study [77]. They are delivered intra-theccally and catalyse the degradation of *HTT* mRNA by RNase H, thereby reducing the production of the mHTT protein (see figure 3). In animal models this results in up to 80% sustained reduction in *HTT* mRNA levels [78].

In RNAi based approaches RNA molecules bind to mRNA in the cytoplasm, prompting its removal by argonaute 2, the RNase enzyme within the RNA-induced silencing complex (RISE) [79], see figure 3. Therapeutic strategies using this approach are currently in the preclinical phase. RNAi delivery is more invasive than ASOs requiring intracranial injection into the striatum. However a single treatment may provide permanent *HTT* lowering [2]. Small molecule splicing modifiers have shown promise in animal models of small muscular atrophy [80] and screening is currently underway to identify small molecule splicing modulators of mHTT [81].

Targeting the DNA of mHTT can be achieved using two approaches, zinc finger proteins and the CRISPR/Cas9 (clustered inter-spaced short palindromic repeats) system. Zinc fingers are proteins forming a structural motif that bind to DNA. Synthetic zinc finger transcription factors targeting CAG have been used to reduce levels of mHTT protein in animal models. However as they create non-native proteins they have the potential to cause immune reactions thus further work is needed [82].

CRISPR/Cas9 is used by bacterial immune systems in order to cleave foreign DNA. In recent years the system has been harnessed as a tool for genome editing with a multitude of applications to human disease. This technology has been used in fibroblasts of a HD patient to excise the promoter regions, transcription start site and the CAG mutation expansion of the mHTT gene. This resulted in permanent and mutant allele specific inactivation of the mHTT gene [83]. Recently the method was successfully tested in an HD rodent model [84]. This affirms the feasibility of this approach but much preclinical work is needed to bring these rapidly-evolving technologies to the clinic, especially given recent concerns about unexpected off-target mutations with CRISPR-Cas9 gene editing [85].

Figure 3. The production of huntingtin protein, and targeted molecular therapies in development to reduce it. Yellow marks the pathogenic expanded CAG tract and its polyglutamine product. Therapeutic approaches are highlighted with pink boxes. Yellow boxes indicate the most widely accepted toxic species. Dotted arrows and grey boxes indicate proposed non-traditional mechanisms for the production of toxic species. The chief mechanisms of action of ASOs and RNAi compounds are shown at the bottom. The image of huntingtin protein is adapted from reference [86] under a creative commons licence ([CC-BY-4.0](https://creativecommons.org/licenses/by/4.0/)). Reproduced with permissions from [2]



Conclusions

HD is a progressive and devastating disease. Over the last decade there has been a rapid growth in our understanding of the natural history of HD and pathogenesis both at the cellular and macroscopic level. To date few treatments are available and a number of clinical trials of failed. However the development of therapeutic strategies capable of targeting mHTT directly heralds a new era for HD research. Now more than ever there is a real potential to modify and potential prevent HD.

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