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Recent Updates on Huntington Disease

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Abstract

Huntington Disease (HD) is an autosomal dominant neurodegenerative disorder and a common cause of mortality. It affects both sexes and is prevalent in all ethnic group globally. The incidence reported is 5-7/100,000 in western population. As the age of onset can range from 2-85 year with majority of the cases being diagnosed between 30-40 years of life, hence it can be grouped as adult-onset disorder. It is a progressive loss in the motor, cognitive and behavioral abilities. MRI scans reveals that mostly neurons of basal ganglia are affected that are involved in muscles helping in movements of body. The main cause of HD is expansion of CAG repeat in the HTT genes beyond the threshold size. Presently, there is no cure for HD and the current treatment includes management of symptoms using antipsychotics and antidepressants. Thus, understanding the pathways and molecular pathophysiology might be helpful in designing the possible leads for drug discovery. The review focusses on current possible drug targets for HD and shed some lights on the use of NGS-based techniques to determine the etiology of HD that can be further used in the therapeutic design for HD.

Keywords: Huntington Disorder (HD); CAG repeat; Neurodegenerative disorder; HTT; mHTT

Introduction

Huntington Disease/Disorder (HD) is a fatal neurologic disorder that affects both the sexes in all the ethnic group and races worldwide. This devastating disease is more common in the western population where it is estimated to affect 5 to 7 individuals per 100,000 [1]. It was in 1872 when this disease was first described in detail by an American doctor George Huntington and was called as Huntington chorea due to the presence of involuntary dance like movements as presented by patients affected with it [2]. With the finding that it is also associated with other symptoms like cognitive and behavioral impairment it became more commonly addressed as Huntington disease. HD is a genetic disorder that follows an autosomal dominant inheritance pattern there by affecting 50% of the offspring in case when one of the parents is the carrier of HD gene [3]. The onset of the disease is quite variable but is typically in the prime of the adult life, being most frequent between 30-40 years of age. However symptoms may also start as early as 2 years of age or as late as 85 years [4]. If the disease symptoms appear before the age of 20 years it is known as Juvenile HD (JHD), Akinetic-Rigid or Westphal Variant HD. But JHD is very rare and happens in only 7% cases. The main clinical features associated with HD includes neurologic symptoms like chorea, dystonia, rigidity, balance problems and eye movement abnormality, psychiatric problems including depression, mania, psychosis, and suicidal tendency cognition related problems like speech difficulty, executive function loss, short term memory loss, attention problems and problem in calculation [5-8]. Though HD is a known neurodegenerative disease but it is found to be associated with many non-neurologic peripheral abnormalities like abnormal energy metabolism, extreme weight loss, osteoporosis, muscle and testicular atrophy, diabetes, and reduced pulmonary function. In fact leading causes of death of HD subjects is not neural death but instead pneumonia and heart disease [9,10]. This suggests a multi-domain involvement of the disease pathology.

Literature Review

Disease progression in HD

HD is a neurodegenerative disease which results in progressive decline in the motor, cognitive and behavioral abilities of the affected person. In early stages of life the person will have full functional abilities and will remain clinically asymptomatic. Later subtle changes in cognition (mostly execution related problems) and psychiatric function may start appearing this is referred to as prodromal stage. Then gradually the symptoms become more obvious, chorea being the most prominent feature. This marks the beginning of manifest stage where the motor and cognitive abilities of the individual become more and more compromised. Early finding of manifest stage includes mild chorea, abnormal extra-ocular movements, brisk muscle stretch reflexes, and diminished rapid alternating movements. However Chorea plateaus off as the disease progresses [11]. Although the depleting functional abilities may have a great psychological impact on the affected person and also it was found that depression is typical for HD and suicide rates are found to be about five to ten times higher than that for the general population. But this depression and suicidal tendency appears to be an integral part of disease process and not as a response to debilitation caused by disease progression as depression and apathy can occur several years before the motor abnormalities begin [12]. The neuropathology of depression is related to impairment in several domains like monoaminergic systems imbalance,

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Hypothalamus Pituitary Adrenal (HPA) axis dysregulation, neuroinflammation, oxidative stress, and a decrease levels of neurotrophins like BDNF and genetic predispositions [13]. Late stage HD is characterized by bradykinesia, spasticity, dysarthria, dysphagia, incontinence, and complete dependence.

Neurobiology of HD

In HD, the major neuropathology is the progressive loss of Medium-sized Spiny Neurons (MSNs) in the striatum of the forebrain. MSNs are responsible for coordinating the muscle driven movements of the body by interacting with the motor cortex via direct pathway and indirect pathway. Due to striatal cell loss in early stages of HD, the interaction between basal ganglia and motor cortex get disturbed thereby resulting in chorea. During the initial stages of the disease, the GABAergic neurons of the indirect pathway (which co-express enkephalin as a secondary neurotransmitter) are primarily affected, leading to an overall disinhibition of the thalamus and hyperkinesia; as the disease progresses the GABaergic neurons of the direct pathway (which co-express substance P and a secondary neurotransmitter) also degenerate, resulting in over-inhibition of the thalamus and hypokinesia/rigidity. Thus, over the course of the disease, HD progresses from being a hyperkinetic to a hypokinetic disorder.

In JHD both striatal and cortical cells are affected in the early stages so chorea is not generally associated with it. Neurodegeneration also occurs in "downstream" structures of the striatum-the globus pallidus, substantia nigra and thalamus. Variable intensity of neural cell loss is also observed in various regions of the cerebral cortex, hippocampus and amygdala of the limbic system. These regions are important for controlling the thought process, emotions memory and learning functions. About 25%–30% reduction in total brain weight is seen in patients with advanced HD. Loss of neurons from different brain regions may lead to physical, emotional and cognitive decline. Below average brain volumes have been found in HD individuals even before disease symptoms appear. Post-mortem morphometric investigation of HD patients showed a 21%-29% area loss of the cerebral cortex, 29%-34% loss of telencephalic white matter, 64% loss in the putamen, and 57% loss in the caudate nucleus, as compared to that of control same age individuals.

Genetic basis of HD

The gene responsible for HD was first identified in 1993 and was called as HTT gene. The HTT gene spans about 180 kb and consists of 67 exons and is located on short arm of chromosome 4 at a location IT15. HD is a triplet repeat disorder that is caused due to expansion of CAG repeat in exon 1 of HTT gene, beyond a threshold number. The CAG repeat is quite polymorphic in size and varies among different individuals.

Depending upon the size of the CAG repeat there are 4 types of HTT allele-normal HTT allele in the range 6 to 26, intermediate alleles with 27–35 CAG repeats, reduced penetrance allele with CAG repeat ranging between 36-39 (may or may not cause HD) and disease causing allele (≥ 39 CAG repeats). These CAG repeats may be interrupted by glutamate encoding CAA repeats. Interestingly, recent evidences showed that patients with no CAA interruptions have early disease onset suggesting that the timing of onset is a property of the expanded CAG repeat rather than the length of polyglutamine, but the underlying mechanism of this toxicity is not clear. The intermediate allele and reduced penetrance alleles show paternal genetic anticipation and thus are at high risk of having off-springs with expanded allele. Weather the person will develop HD or not that solely depends upon the length of the CAG repeat in expanded allele and has no contribution of the CAG repeat length of other allele either normal or expanded. CAG repeat length is inversely correlated with the age of onset and age of death in affected individuals. However the mean duration of the disease remained same for those with small or large CAG repeat length. This is evident with the fact that 40-50 CAG repeat is related to adult onset HD while those with JHD have more than 60 repeats. But CAG repeat length show approximately 60% variation with the age of onset, this is thought to be due to presence of functional genetic differences (genetic modifiers) that may interact with the mHTT during different stages and there by resulting in differences in onset, manifestation and progression of the disease in different individuals. A recent genome wide association study on 4000 HD patients recognized a chromosome 15 locus-two independent effects that accelerate or delay onset by 6.1 years and 1.4 years, respectively, and a chromosome 8 locus hastens onset by 1.6 years. Similarly, DNA damage response related pathways have known to modulate expansion and pathogenicity of the repeat tracts in HD, which in turn alters the disease presentation. This suggests, that studying genetic modifiers in HD will aid in identifying pathways that are critical in disease manifestation and therefore could form ideal avenues for designing better drug targets to delay or prevent onset of HD.

Pathophysiology of HD

The HTT gene encodes for Huntingtin protein (HTT) whose exact function is ill defined however it is believed to be important for the nervous system development. HTT is expressed ubiquitously throughout the body with highest levels in the brain and testes. HTT protein can interact with a large number of proteins with known and unknown function. It is found to be involved in trafficking of various proteins, transport of vesicles, clathrin mediated endocytosis; synaptic signaling, transcriptional regulation, and anti-apoptotic function. A loss of function of the HTT and a toxic gain of function of the mHTT thus leads to the disturbance of multiple cellular pathways.

The CAG repeat expansion mutation in HTT gene results in production of abnormally long stretch of Poly glutamine (Poly Q) tract in the mutated HTT (mHTT) protein that may have altered functionality. Thus either due to loss of function of normal HTT or gain of toxic function of mHTT results in neural cell death. Toxic mHTT fragment could be generated either due to proteolytic cleavage of mHTT or as a result of alternative splicing during transcription that yielded mHTT fragment containing the exon 1, which is thought to be the most toxic. These fragments might interact with each other to form aggregates which is the hallmark for HD. Clarity regarding the role of aggregates is still controversial. It is suggested by some researchers that aggregate formation is a way *via* which the cell attempts to sequester toxic HTT monomers that might be harmful for cellular function. This theory is supported with the finding that large Inclusion Bodies (IBs) have shown to decrease cellular mHTT level and increase neural survival. Also Shortstop, a HD mouse model expressing the first two exons of mHTT with an expanded poly Q repeat, showed no evident neurodegenerative phenotype. However many studies have shown implications of mHTT monomer and/or oligomers in neurotoxicity. Sanchez, et al., reported that blocking poly Q-mediated associations has a neuroprotective effect. The possible explanation could be that even if inclusions are not responsible for toxicity initially and is a way in which cell tries to cope with

accumulating monomeric and oligomeric toxic HTT, but as they keep on accumulating they sequester other vital proteins with important functions and disrupts the function of important cellular organelles in the cell like mitochondria. Also these aggregates cause impairment of the protein quality control and degradation machinery leading to disease pathogenesis. In fact the protein homeostasis is found to be impaired in HD as evident from the accumulation of ubiquitin positive inclusions in the brain tissue of HD patient suggest that these aggregates are targeted for proteolysis but are resistant to removal. Also the HSP70 and DNAJ chaperones levels are progressively reduced in brain tissues of HD mice. Both these findings are suggestive of aggregation process to be involved in neurotoxicity in HD. Thus targeting the growth of these aggregates and their maturation represents a potential therapeutic strategy.

Apart from this general consideration there are other major molecular pathway that may get altered due to presence of mHTT. Mitochondrial dysfunction has been enormously studied in context with HD pathogenesis. mHTT interacts with the mitochondrial outer membrane and cause impairment of Electron Transport Chain (ETC) complexes II and III, thereby decreasing intra-cellular ATP pool and increasing Reactive Oxygen Species (ROS). Mitochondrial dynamics is also found to be perturbed with an excessive mitochondrial fission taking place in HD as evident from presence of fragmented and structurally damaged mitochondria in HD brain. Mutant HTT has been found to interact with Drp1 and elevate GTPase Drp1 enzymatic activity in postmortem HD brains which is involved in mitochondrial fission. Also mHTT interacts with and impairs PPAR-γ co-activator-1α (PGC-1α) that regulates expression of gene in mitochondrial biogenesis.

Proper trafficking of mitochondria to neuronal ends are important as these are the places where synaptic connections takes place and there is high energy demand. But in HD the axonal transport of mitochondria is also impaired either due to interaction of mHTT with HAP (Huntingtin Associated Protein) that is involved in intracellular trafficking or due to physical hindrance caused by mHTT aggregates in the narrow region of the neurons. These findings are in line with the compromised energy production and metabolism as observed in HD patient. Thus it seems that mHTT may either indirectly affect mitochondrial functions via transcriptional dysregulation of genes important for mitochondrial biogenesis or can directly interact with and can adversely affect mitochondrial movement and functions resulting in HD phenotype.

Gene expression profile in case of HD is highly altered such that expression of mHTT has global effect on the transcriptome. Soluble mHTT oligomers sequester and thus impede the function of important proteins that are important for general promoter accessibility and transcription initiation like Specificity Protein 1 (SP1), TATA box Binding Protein (TBP), the TFIID subunit TAFII130, the RAP30 subunit of the TFIIF complex, and the CAAT box transcription factor NF-Y. Sequestration of CBP (CREB Binding Protein), that has a histone deacetylase domain, alleviates expression of many genes important for proper synapse development and memory. mHTT also facilitates Polycomb Repressive Complex 2 (PRC2), mediated methylation of histone H3 in lysine 27 and causes transcriptional repression. A decrease in expression of BDNF, an essential neurotrophin of central nervous system is also documented in tab HD. This is a result of incapability of mHTT in binding REST which is transcriptional co-repressor of BDNF. Usually REST interacts with wild type HTT and stays in the cytosol. As mHTT fails to bind REST, it translocates to the nucleus and binds the Repressor-Element 1 (RE1) blocking BDNF gene transcription. These finding implies transcriptional dysregulation to be significantly involved in pathogenesis of this untreatable disease.

Current priority: Therapeutic targets under investigation for HD

PubMed search using keyword Huntington disorder yielded 22874 hits while that using Huntington disorder and treatment yielded 9159 hits and that by using Huntington disorder and therapeutics gave only 7152 results. This depicts that, despite of a plethora of scientific studies and review work done on HD and its pathogenesis not much progress has been made in direction of its treatment.

Till date no clinical trial successfully identified disease-modifying treatments for HD there is no cure for Huntington disease. A list of ongoing clinical drug trials for HD is given in Table 1. Current treatments are therefore primarily symptomatic and include antipsychotics and antidepressants. Supportive therapy involving many forms of medical and social care is suggested in order to lessen the impact of symptoms on the patient. An understanding of the various pathways and cellular events that are disrupted in HD might be helpful in designing possible drug targets. But this is quite challenging in case of HD owing to the fact that precise function of HTT is still not clear and it interacts with several proteins and is involved in plethora of pathway. The question that has to be considered with care is that which of these pathways and molecular events have a pivotal role in the pathology and how many of these processes should be blocked to prevent HD pathology and what molecular tools can be used to test these possibilities?

S. no.	Status	Study title	Clinical trial identifier	Interventions
1	Active, not recruiting	Pridopidine's outcome on function in Huntington Disease, PROOF-HD	NCT04556656	Drug: Pridopidine Drug: Placebo
2	Active, not recruiting	Clinical extension study for safety and efficacy evaluation of Cellavita-HD administration in Huntington's patients.	NCT04219241	Biological: Cellavita-HD
3	Active, not recruiting	Safety evaluation of Cellavita HD Administered intravenously	NCT02728115	Biological: Cellavita HD lower dose and higher dose

		in participants with Huntington's disease		
4	Recruiting	Study of WVE-003 in patients with Huntington's disease	NCT05032196	Drug: WVE-003
5	Recruiting	Impact of Deutetrabenazine on functional speech and gait dynamicsin Huntington disease	NCT04713982	Drug: Deutetrabenazine
6	Recruiting	Safety and Proof-Of-Concept (POC) study with AMT-130 in Adults with early manifest Huntington disease	NCT04120493	Genetic: Intra-striatal rAAV5-miHTT Other: Imitation (sham) surgery
7	Not yet recruiting	A study to evaluate the effect of SAGE-718 on cognitive function in participants with Huntington's Disease (HD)	NCT05107128	Drug: SAGE-718 Drug: Placebo
8	Not yet recruiting	Trial of the combined use of Thiamine and Biotin in patients with Huntington's disease	NCT04478734	Drug: Moderate and high doses of Thiamine y biotin
9	Active, not recruiting	An open label study of ANX005 in subjects with, or at risk for, manifest Huntington's disease	NCT04514367	Drug: ANX005
10	Recruiting	Symptomatic therapy for patients with Huntington's disease	NCT04071639	Drug: Haloperidol 2 Mg tab Drug: Risperidone 1 Mg tab
11	Active, not recruiting	Safety and efficacy of Fenofibrate as a treatment for Huntington's disease	NCT03515213	Drug: Fenofibrate Drug: Placebo
12	Active, not recruiting	An open-label extension study to evaluate long-term safety and tolerability of RO7234292 (RG6042) in Huntington's disease participants who participated in prior Roche and Genentech sponsored studies	NCT03842969	Drug: RO7234292 (RG6042)
13	Active, not recruiting	A study to evaluate the efficacy and safety of intrathecally administered RO7234292 (RG6042) in participants with manifest Huntington's disease	NCT03761849	Drug: RO7234292 Drug: Placebo
14	Recruiting	A dose range finding study with open-label extension to evaluate the safety of oral LMI070/Branaplam in early manifest Huntington's disease	NCT05111249	Drug: Branaplam Drug: Placebo
15	Recruiting	Open-label rollover study for continuing Valbenazine Administration for the treatment of chorea associated with Huntington disease	NCT04400331	Drug: Valbenazine
16	Recruiting	A pilot study assessing impulsivity in patients with Huntington's disease on Xenazine (Tetrabenazine)	NCT02509793	Drug: Tetrabenazine
17	Recruiting	Evaluating the efficacy of Dextromethorphan/Quinidine in treating irritability in Huntington's disease	NCT03854019	Drug: Dextromethorphan/ quinidine 20 mg/10 mg (DM/Q20 mg/10 mg) Drug: Placebo

18	Not yet recruiting	Testing Metformin against cognitive decline in HD	NCT04826692	Drug: Metformin Drug: Placebo
19	Recruiting	Risperidone for the treatment of Huntington's disease involuntary movements	NCT04201834	Drug: Risperidone Device: BioStamp point device
20	Not yet recruiting	Efficacy of Deutetrabenazine to control symptoms of dysphagia associated with HD	NCT04301726	Drug: Deutetrabenazine oral tablet Drug: Placebo oral tablet

Table 1: List of current drug clinical trials of Huntington disorder.

Targeting mitochondrial dysfunction in HD

Many studies have been carried out in an attempt to find possible drug target for Huntington. Drug targeting mitochondrial dysfunction have been recently reported to hold promising results. Treatment with selective inhibitor (P110-TAT) of the mitochondrial fission protein DRP1 reduced mitochondrial dysfunction, motor deficits, neuropathology, and mortality in HD mouse model and fibroblasts and iPS cell–derived neurons from HD patients. The PPAR agonist Rosiglitazone (RSG) is reported to induce mitochondrial biogenesis and prevent mitochondrial dysfunction in cells expressing mHTT. Similarly KD3010 which has been previously used for diabetes trials have showed significant improvements in motor function, neurodegeneration, and survival in the HD mice and also reduced HTT-induced neurotoxicity in medium spiny-like neurons generated from human HD stem cells. Mitochondrial accumulation of mHTT alters the electron transport chain and its nuclear accumulation alters the gene expression and translation of HTT. It was previously reported that N17 domain of HTT is the key regulator of its stability and localization and is implicated in the mHTT mediated pathogenic mechanism. Interestingly, a recently conducted research in zebrafish depicted that mHTT without N17 domain showed an early onset of symptoms, whereas that with N17 domain showed a delayed onset of symptoms. This emphasizes that the expression of N17 domain in mHTT alleviates its accumulation and toxicity and hence N17 domain could serve as a viable target for the development of the new therapeutic strategy for HD.

Targeting impaired histone acetylation in HD

As stated before mHTT disrupts histone acetylation by CBP and thus causes hypoacetylation and silencing of important neuronal genes. This loss of HAT activity may be counteracted by inhibiting HDACs. HDAC inhibitors have shown to ameliorate mHTT toxicity in flies and mice model of HD. Also treatment with HDAC inhibitor may indirectly help in mHTT aggregate clearance by enhancing expression of UPS related genes and by directly increasing acetylation of mHTT at lysine 444 that facilitates trafficking of mHTT into autophagosomes leading to protein clearance and reversing the mHTT toxicity in striatal and cortical neurons in HD model.

Targeting mHTT aggregation in HD

Other approaches to clear off mHTT aggregates have also shown promising results. These include autophagy enhancement by inhibiting mTOR (mammalian Target of Rapamycin) that inhibits autophagy. Recently, two novel brain penetrant compounds-the mTORC1/2 inhibitor PQR620, and PQR530 were reported to induce autophagy without affecting cell viability and reduce mHTT levels in cell models of HD. However mTOR is involved in other important cellular pathways too, thus targeting mTOR may disturb normal cellular functioning. Alternatively, other small molecule enhancers like SMER 10, SMER 28 and SMER 18 have been used to boost autophagy independent of mTOR in cellular and fruit fly model of HD. PolyQ aggregation was inhibited both in vivo and *in vitro* by treatment with a disaccharide trehalose. It has shown beneficial effects on striatal atrophy, weight loss, survival and motor function in R6/2 transgenic mouse model. Furthermore, Poly (trehalose) nanoparticles depicted inhibition of polyQ aggregates under extra/intracellular conditions, reduction of cytotoxicity, and prevented aggregation in a HD mouse brain. Studies have revealed that molecular chaperones alleviate neurodegeneration through modulation of the aberrant protein interactions by mHTT in the early stage of aggregation. Infact, a recent study depicted that allosteric activation of Hsp70 chaperone with a pharmacological mimetic of the Hsp70 co-chaperone Hip, YM-1, was able to reduce the N-terminal huntingtin clustering and nuclear aggregation and thus can modulate huntingtin proteostasis. The eukaryotic chaperonin TCP-1 Ring Complex (TRiC, also called to Chaperonin Containing TCP-1 (CCT)), a member of HSP60 family, have been proposed to protect against polyQ aggregation. Recently, it was established that even exogenous apical domain of a single mammalian chaparonin TRiC/CCT subunit CCT1 (ApiCCT1) when delivered to striatal neuronal cells from full-length knock-in HD mice, can alleviate mHTT-mediated toxicity and can delay the onset of inclusion body formation by sequestering N17 portion of mHTT and thus not allowing it to form aggregates. TRiC/CCT is degraded by ubiquitin proteasome system and the kinase activity of Vaccinia-Related Kinase 2 (VRK2) facilitates its degradation thus it was suggested by Kim, et. al, in 2013 that VRK2 inhibitors can also be utilized for HD therapy. For example, recently, it was reported that neuronal heterogeneous Nuclear Ribonucleoprotein Q (HNRNP Q) specifically binds to the 3'untranslated region of VRK2 mRNA in neuronal cells and reduces its stability, which in turn suppresses polyglutamine huntingtin aggregation in human neuroblastoma cells and mouse cortical neurons, hence preventing HD.

Excitotoxity has long been implicated in HD pathogenesis. Perturbation in Kynurenine pathway is reported in HD model. This is an important pathway for tryptophan degradation in glial cells of the CNS. The enzyme Kynurenine Monooxygenase (KMO) is a key branchpoint in KP (Kynurenine Pathway), and when its activity decreases Kynurenic Acid (KA) is produced which is neuroprotective while neurotoxic Quinolinic Acid (QA) is produced when KMO is more active.

The level of QA is comparatively high than KA levels in post-mortem HD patient brain. Researchers have observed that inhibition of KMO *via* CHDI-340246, increases the levels of KA in HD rodent models and in Cerebro Spinal Fluid (CSF) of nonhuman primates and thus appears promising to cure HD. Of late a study has identified 19 new KMO inhibitors, one of them (named as 1) is found to be neuroprotective in a Drosophila HD model however it is minimally

brain penetrant in mice. The prodrug variant (named as 1b) has shown to cross the blood–brain barrier and release KMO inhibitor 1 in the brain, thus lowers the levels of 3-hydroxykynurenine, a toxic KP metabolite associated with neurodegeneration. Hence this study revealed that, prodrug 1b will lead to advance development of targeted therapies against KP pathway related neurodegenerative diseases including HD. The said excitotoxicity in HD may also be a result of excess amount of excitory neurotransmitter glutamate at the synapse that cause NMDA receptor activation and hence raises intracellular calcium level. High intracellular calcium concentration leads to activation of cellular degradation processes involving proteases, lipases, nitric oxide synthase causing cell death. This is evident from the finding that the NAMDA receptor currents is enhanced in both *in vivo* and *in vitro* HD models. Also the expression of Excitatory Amino Acid Transporter 2 (EAAT-2) that is responsible for removing extracellular glutamate, is found to be compromised in HD mice and HD patient brains. Therefore drugs that enhance the activity or expression of EAAT-2 may be explored to show beneficial effect in controlling neural death in HD.

Targeting neural cell death

Neural cell survival is also compromised in HD due to decrease in level of an important neurotrophin BDNF owing to its reduced expression and axonal transport. Numerous studies focusing on pharmacological interventions for increasing endogenous BDNF levels are done using *in vivo* HD models. These strategies would circumvent the problems related to the use of invasive methods of BDNF delivery while allowing for the correct dosage and stability of this neurotrophin. Treating both HD transgenic mouse models and human HD patients with riluzole, a non-competitive inhibitor of ionotropic glutamate NMDA receptors demonstrated an increase in BDNF expression and amelioration of HD symptoms. Administration of antidepressant, sertraline in R6/2 mice model of HD resulted in an improvement in motor performance; decrease in striatal atrophy, and in prolonging surviva. In the same mice model adenoviral mediated delivery of BDNF in the striatum prompted striatal neurogenesis. Recently, immunomodulators, like Laquinimod and Glatiramer acetate have shown to elevate BDNF levels and positively impact motor function in HD mouse model in independent studies. Targeting BDNF signaling might also improve non-motor deficits like long term memory, depressive-like and anhedonic behaviors and cognition related features in HD mouse model. Binding of BDNF to TrkB receptor mediates differentiation, proliferation, survival, migration and branching. Thus in absence of BDNF alternative modalities that would activate TrkB receptors might be useful. Recently, monoclonal antibodies specific for TrkB depicted promising results in protection of striatal neurons from mHTT-induced cell death in HD models. However delivering these antibodies to the striatum might be challenging.

Targeting mHTT expression in HD

An advancement in technology have led to emergence of approaches that relies on lowering the expression of mHTT at the level of DNA (transcription) or RNA (translation) with an insight that this will reduce all of the downstream deleterious effects of the mutant protein that lead to the manifestations of HD. These include RNA interference (RNAi) using short interfering RNA (siRNA); (shRNA); translational repression using single-stranded DNA based Antisense Oligonucleotides (ASOs); and transcriptional repression using Zinc Finger Proteins (ZFPs).

Intrastriatal injection of Adeno-Associated Virus (AAV2) vector expressing HTT-silencing miRNA in YAC128 HD mouse model resulted in a transduction of approximately 80% of the striatum and approximately 50% reduction is noted in HTT mRNA and HTT protein and mHTT aggregation. Though both wild type and mutant Huntington levels were found to be reduced still there was no evidence of inflammation or neurotoxicity reported, while the performance of treated mouse improved. Similarly, intrastriatal administration of AMT-130 resulted in potent and sustained suppression of HTT in Hu128/21 mice for at least 7 months post-injection and leads to improvements of behavioral and neuropathological phenotypes. Furthermore, this gene therapy in induced-Pluripotent Stem Cells (iPSC) derived from neurons and astrocytes from 2 patients carrying different HTT mutations showed that the level of HTT was lowered by 68% in a single dose, while there was no evidence of off-target effects.

Moreover, artificially synthesized ASO's are of lot of interest currently after the successful first phase 1 human trials of ASO targeting Superoxide Dismutase 1 (SOD1) in familial amyotrophic lateral sclerosis in 2013 that was completed without significant safety issues. Thus paving way of such trials in HD also. In fact ASO-HTT-RX developed by Isis pharmaceuticals and Roche have shown successful results in animal models and in phase 1 clinical trial. However, as the study was monitored in phase III clinical trial it was subsequently brought to halt in March 2021, as there were no recognizable clinical effectiveness of the drug and/or the drug appeared less safe than expected.

The main concern in application of these approaches is to specifically target HTT allele. HTT allele specific sequence differences in the wild type and mutant allele could be exploited for this. A deletion mutation Δ2642 that removes one among 4 GAG repeats in exon 58 of the HD gene is found to occurs more frequently in mHTT alleles (38%) than in wild-type HTT (7%). A study on Caucasian 234 HD patients identified fifty HD-SNPs across the HTT gene that are significantly enriched on HD alleles compared to wild type alleles and about 85% of HD patients can be covered by targeting as few as three SNPs. These basic sequence differences might be targeted to develop allele specific silencing methods for HD. Other major concern is the delivery method. siRNA are incapable of crossing the blood brain barrier thus limiting its therapeutic potential. However but this can be enhanced by a number of methods, including viral vectors, exosomes, cholesterol conjugation, convection-enhanced delivery, and novel conjugates of single-stranded siRNA compounds. ASO can be delivered directly in the CSF owing to its smaller size and chemical structure and was found to elicit therapeutic effect up to 4 month thus giving huntington holiday to the brain. Another type of small non coding RNA that could be used as a therapeutic strategy for HD are short hairpin RNAs (shRNAs). In contrast to siRNAs, shRNAs based approaches, depicts lesser toxicity due to fewer "off-target" effects and has a long-lasting effect.

However, technical challenges are present related to shRNA overexpression. For instance, an intracellular overexpression of shRNA targeting the mHTT mRNA leads to clogging of endogenous miRNA processing thereby causing toxicity. Likewise, a study by MCBride, et al., identified that shRNAs targeting conserved sequences in human HD and mouse HD homolog (HDh) mRNAs caused a reduction in HDh mRNA expression in a knock in HD mouse

model. However, two of the three shRNA studied induced significant neurotoxicity in mouse striatum. The two shRNAs which showed toxicity also generated higher antisense RNA levels, as compared to the nontoxic shRNA. This demonstrated that overexpression of antisense RNAs can be problematic in the mouse brain. These toxic shRNA sequences when placed into artificial microRNA (miRNA) expression systems, then the neurotoxicity were significantly weakened while not compromising mouse HDh silencing efficiency. Another study also depicted similar relation between, *in vivo* shRNA overexpression to tissue damage and recurrent lethality in adult animals. The findings of this study revealed that a toxic intracellular shRNA threshold level is linked to a fatal competition with endogenous miRNA processing and functionality. The authors suggested that by monitoring and regulating intracellular shRNA levels, stable *in vivo* gene silencing could be obtained, while alleviating adverse effects. Once, the technical challenges related to shRNA based therapy could be solved, it may surely be used as a powerful approach to combat HD and other related neurodegenerative disorders.

Another gene silencing approach which is more in the pipeline is the use of ZFP that are artificially engineered proteins that specifically bind to the defective expanded HTT DNA sequences (but not the normal HTT sequences or other, unrelated sequences) and repress the synthesis of the toxic gene products. ZFP target the origin that is the defective gene rather than RNA. As mHTT RNA might also have certain deleterious effects which are not yet known thus by targeting the gene itself the production of toxic RNA can be avoided. This approach looks promising as the problem of generation of alternative mHTT fragments not containing target site of siRNA will not be encountered. In HD mouse model 60 percent reduction of the HD protein and improved symptoms was observed on treatment with ZFP. Recently, a study depicted that virally delivered ZFP-Transcription factors selectively repressed >99% of HD-causing alleles over a wide dose range in patient-derived fibroblasts and neurons, while the expression of >86% of normal alleles was preserved. Also other genes with CAG repeats were least affected. This study showed that the ZFP-TFs remained active and were well tolerated in HD neurons for more than100 days in culture and for at least 9 months in mouse brain. This seems to be very promising still a lot of technical improvement is required before it could be replicated in human trials.

Targeting neural stem cells for HD

Cellular therapies can be prospectively used for restoring atrophied tissues and thus provides important therapeutic possibility. Stem cell transplantation strategy for HD treatments can be utilized for replacing the dysfunctional or lost neurons. For this methods for obtaining the optimal Neural Stem Cells (NSCs) are to be developed from various sources, like brain, Pluripotent Stem Cells (PSCs), and somatic cells of the HD patients. Stem cell-based therapy has been successfully applied in HD animal models and functional recovery has been reported by various studies. By genetically engineering stem cells to over-express neurotrophic factors or preconditioning those with compounds that can stimulate the production of neurotrophic factors their effect can be enhanced. For instance, a recent study demonstrated that intra-cerebral transplantation of BDNF-overexpressing human NSC (HB1.F3.BDNF) into the contra-lateral side of unilateral Quinolinic Acid (QA)-lesioned striatum promoted migration, differentiation and functional restoration in HD rat model. Similarly, Intranasal delivery of bone marrow-MSCs (Mesanchymal Stem Cells) (preconditioned with mood stablizers) in a N171-82Q HD transgenic mice lead to functional improvements, reduction in neuropathological features, reduction in striatal neuronal loss and ameliorated huntingtin aggregates. Another study conducted on Huntington monkeys utilized Dental Pulp Stem/Stromal Cells (DPSCs) and demonstrated that they are a potential source of personal stem cells with less post-transplant immune rejection and thus can be used for therapeutic purposes in HD. Transplanting MSCs derived from the Umbilical Cord (UC) are an attractive alternative as the latter is a non-controversial, inexhaustible source of stem cells and can be harvested at a relatively lower cost. These UC MSCs are an intermediary connection between adult and embryonic tissue thus have greater pluripotency than adult stem cells which are derived from other sources. In this line, UC-MSCs, isolated from day 15 gestation pups, were transplanted intrastriatally into 5-week-old transgenic R6/2 mouse model of HD and a transient improvement in a spatial memory task was observed with the finding that, grafting of high passaged UC MSCs provided greater behavioral and neuropathological sparing than low passaged UC MSC. Of late, a study targeted *SUPT4H1* gene which selectively supports transcription of long trinucleotide repeats. Here, SUPT4H1-edited HD-induced pluripotent stem cell-derived neural precursor cells (iPSC-NPCs) were transplanted into the YAC128 HD mouse that lead to a reduction in mutant HTT expression without compensating the wild-type HTT expression, and caused an improvement in the motor functionality in comparison to unedited HD iPSC-NPCs.

Discussion

Overall, stem cell based therapies offer promising opportunities for the treatment of HD. Still, it is vital to recognize various sources of stem cells that give optimal outcomes before it could be clinically translated. Also, more in-depth and comprehensive pre-clinical studies using rodent and non-human primate HD models will be needed to confirm its therapeutic potential.

Advances in therapeutics for HD has been recently reviewed by Kim, et. al., and has elaborated on approved treatment options and current clinical trials for HD. This review is quite extensive however an important recent finding which reported the development of first human genomic transgenic mouse model of HD with long uninterrupted CAG repeats could not be incorporated. This study for the first time compared new BAC-CAG model with long uninterrupted CAG repeats, with the previous HD model with frequent CAA interruptions. The result depicted that the long CAG repeat is selectively toxic to striatum following distinct toxic molecular mechanisms that are originated from the expanded uninterrupted CAG repeat locus *viz*. somatic DNA repeat instability, RNA gain-of-function toxicities and toxic protein products due to repeat-associated non-ATG translation (RAN translation). Thus emergence of this new model is matchless from a therapeutic perspective and with the growing understanding of the HD pathophysiology and technical advancement we may soon be entering in an era when HD would be curable or at least its onset and progression could be delayed.

Biomarkers in HD

The word "biomarker," a portmanteau of "biological marker," refers to a large subclass of medical indicators, that is, objective indications of medical status observable from outside the patient, which can be evaluated reliably and reproducibly. These biomarkers may be useful in establishing endpoints in clinical trials in this group. Current advances in the discovery of biomarkers may enable the measurement of disease progression in people at the premanifest stage of the disease.

Researchers are looking at the significance of clinical, cognitive, neuroimaging, and biochemical biomarkers for the development of upcoming therapies for people with Huntington's disease. In therapeutic trials for HD, the United Huntington's Condition Rating Scale (UHDRS) is used to gauge patient response to treatment and track the course of the disease. It is a set of measures intended to identify clinical alterations in HD that is apparent. As a result, the UHDRS might not be sensitive enough to identify the subtle characteristics present in certain pre-manifest people, especially those who are only a few years away from manifesting the condition. Molecular imaging indicators for MR and PET have been created as tools to track the development of the illness and assess the effectiveness of disease modifying therapies in HD. For the purpose of identifying structural and pharmacologic alterations that could be brought on by the reduction of mHTT, these markers may be utilized singly or in combination. Lowering brain levels of the mutant Huntingtin (mHTT) protein is the main objective of new HD studies in an effort to slow or stop the disease's development. Wet biomarkers, or those derived from body fluids, are yet another potential source of helpful outcome measurements, especially if they represent the pathobiology of the disease. Many different pathogenic pathways have been connected to HD, leading to a large number of possible molecular markers. In HD, NF-L levels are associated with motor and cognitive deterioration as well as brain shrinkage. In comparison to mHTT levels, NF-L distinguishes between presymptomatic and symptomatic phases more effectively and exhibits strong relationships with clinical aspects of HD and brain volumetrics. In addition, in presymptomatic individuals, plasma NF-L predicts the age of illness start in the subsequent three years. By themselves, HTT and mHTT can be regarded as biomarkers. Despite having a predominate intracellular localization, HTT can be physiologically secreted into the CSF, as well as mHTT in pathological conditions, as a result of the removal of brain extracellular solutes by the glymphatic system (an aquaporing-4 water channel-based waste drainage system), as demonstrated in animal models. The concurrent use of both protein tests offers obvious benefits in monitoring progression and discovering possible treatment effects due to the synergistic function of mHTT and NFL in the development of HD. One of the most often utilized indicators of oxidative damage to nucleic acid is the DNA oxidation product 8-hydroxydeoxyguanosine (8-OH d g). In the PREDICT-HD research, leukocyte 8O Hd g was demonstrated to be a very sensitive biomarker of Huntington's disease. It is unclear if this represents greater oxidative stress, decreased turnover or repair of oxidized bases in HD, or something else related to disease presence or progress. Numerous research teams have used the redox biomarkers of DNA, proteins, and lipids that are currently available, such as protein carbonyls, 3-nitrotyrosine, malondialdehyde, 3-hydroxykynurenine, 3-hydroxy-anthranilic acid, and TBARS, etc., to look into the role of oxidative stress in various HD models. PDE10A is primarily expressed in the brain (with the striatum having the highest expression), and it becomes profoundly dysregulated in the basal ganglia regions of HD mouse models and postmortem brains of patients. This may help to explain the deficiencies in cAMP-dependent signaling and related CREB transcriptional activity. PDE10A concentrations in the brains of HD patients may be observed by positron emission tomography by using particular radioligands ((18F) MNI-659 and (11C) IMA107) (PET). The presented investigations showed changes in this phosphodiesterase's levels that might occur in pre-symptomatic phases before overt brain shrinkage and associated with numerous indices of disease progression with greater accuracy compared to volumetric assessments. Using a proteomics method, it was discovered that inflammatory-related proteins, such as complement elements, 2-macroglobulin, and clusterin, were changed in the plasma of expansion carriers. Proinflammatory IL-6 levels were shown to be higher in the plasma of intermediate HD patients in comparison to early, premanifest, and control donors in the same investigation. Increased levels of IL-6 were found in premanifest carriers, with an estimated average of 16 years before the age of motor manifestation, according to a separate investigation. MicroRNAs (miRNAs) are of particular importance as biomarkers as they may be isolated from plasma and serum fractions in addition to whole blood as cellular transcripts. Certain miRNAs (miR-122-5p, miR-100-5p, miR-641, and miR-330-3p) have been linked to patient functional skills in the case of HD and alterations in circulating miR-10b-5p and miR-486-5p in plasma mimic those seen in brain miRNAs. But the repeatability and validation of mRNAs remain problems for these RNA types as well. A growing number of biomarkers have been put out in recent years to forecast the age of start and severity of the illness in HD. Due to the low frequency of the condition, validation of these candidates has often been impeded, making it challenging to recruit relatively homogenous cohorts.

Conclusion

HD is a fatal condition that worsens with time and affects both the proband and their family. It has an impact not only physically but also psychologically and socially on individuals who experience it. Many articles have surfaced that have advanced our understanding of the illness, including its pathophysiology and natural history as well as patient management, which has improved over the past 20 years. The pathogenesis and development of HD are influenced by a variety of factors. A lot of clinical trials have failed, and there are currently few medicines available. Preclinical research is now being done on HTT-reducing gene-editing methods such as CRISPR-Cas9, transcription activator-like effector nucleases, and zinc finger proteins. However, these methods need the injection of viral vectors needs more regulations and trail before therapeutic use. Cerebrospinal fluid and serum biomarkers, such mutant HTT and neurofilament light chain, can predict and monitor the course of the illness and are among the earliest detected abnormalities in HD.

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