

S.I. : Genetic pathways to Neurodegeneration

Huntington's disease: the coming of age

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Abstract

Huntington's disease is caused to an abnormal expansion in polyglutamine repeats in the first exon of huntingtin gene. The mutation in huntingtin causes abnormalities in the functioning of protein leading to deleterious effects ultimately leading to the demise of specific neuronal cells. The disease is inherited in an autosomal dominant manner and leads to a plethora of neuropsychiatric behavior and neuronal cell death mainly in striatal and cortical regions of brain eventually leading to death of the individual. The discovery of the mutant gene led to a surge in molecular diagnostics of the disease and in making different transgenic models in different organisms to understand the function of wild type and mutant proteins. Despite difficult challenges there has been a significant increase in understanding of the functioning of the protein in normal and other gain of function interactions in mutant form. However, there have been no significant improvements in treatments of patients suffering from the ailment and most of the treatment is still symptomatic. Huntington's disease warrants more attention towards better understanding and treatment as more advancement in molecular diagnostics and therapeutic interventions are available. Several different transgenic models are available in different organisms ranging from fruit flies to primate monkeys for studies on understanding the pathogenicity of the mutant gene. It is the right time to assess the advancement in the field and try new strategies for neuroprotection using key pathways as target. The present review highlights the key ingredients of pathology in the Huntington's disease and discusses important studies for drug trials and future goals for therapeutic interventions.

Huntington's disease (HD) is a progressive neurodegenerative disorder, which usually manifests in adulthood and is inherited in an autosomal-dominant manner. The disease has peculiar phenotypes with distinct motor defects, psychiatric symptoms and cognitive decline. The motor symptoms include chorea (bizarre dance like movements), dyskinesia (difficulty or distortion in performing voluntary movements) and dystonia (involuntary contraction of muscles). Psychiatric symptoms, that usually precede the motor symptoms, include depression, anxiety and sleep disorders (Spires and Hannan 2007). Cognitive decline is manifested as difficulties in concentration and retaining newly acquired information, decline in language skills, disorganized speech and perceptual impairments. As the disease progresses, motor rigidity and dementia predominate. Several brain areas show signs of neuropathology in HD, with the maximum degeneration occurring in the caudate nucleus and putamen (Vonsattel *et al.* 1985).

The mutation in huntingtin (*htt*) gene was identified in 1993 as an unstable expansion of CAG (the trinucleotide coding for glutamine) repeats, which occurs within the first exon of the gene "IT 15" (for "Interesting Transcript 15") in HD patients (The Huntington's Disease Collaborative Research Group 1993). This gene on chromosome 4 (4p63) encodes the protein huntingtin. The discovery of mutant *htt* was one of the single most major events in the history of HD and genetics. It led to a hurricane of events starting from an efficient molecular diagnosis of HD to the development of mutant *htt* knock-in transgenic models of the disease.

The mutation in huntingtin gene produces an expanded stretch of the amino acid glutamine (denoted by the letter Q) towards the amino terminal end of the protein. This causes the mutant protein to interact abnormally with other cell proteins, leading to changes in its function. The glutamine tract in *htt* is polymorphic, with 8 to 36 glutamine repeats in the normal population and 41 or more in HD. People with 38 or more glutamine repeats in *htt* can manifest HD but occasional cases of people with upto 41 repeats living into old age with no discernible symptoms have been reported (Rubinsztein *et al.* 1996). Several studies have pointed out a strong inverse correlation between the repeat number and the age at onset of HD (Zühlke *et al.* 1993; Nørremølle *et al.* 1993; Tabrizi *et al.* 2013).

Historical Background of HD

A vivid account of the disease by George Huntington has subsequently borne his name and is considered to be the best report and landmark in the study of HD (Huntington 1872). All the cardinal features of HD are listed in the description: the adult onset, progressive course of the disease, choreiform movements, mental impairment, suicidal tendency and the pattern of inheritance. Such a vivid description of the disease was made possible because of the clinical observation of HD patients by Huntington, his father and grandfather. The clear study and documentation of the two older generations of the Huntingtons gave George Huntington a unique advantage in describing the disease fully.

In 1883, Westphal described juvenile symptoms resembling those seen in HD but he attributed it to a cause other than HD as the patients showed a predominance of hypokinesia and rigidity. The term 'Westphal variant' is often used to describe the clinical picture of juvenile HD. Although there were several reports about the neuropathology of HD, it was not until the 1920s that there was an equivocal agreement that in HD the brain changes are primarily degenerative and atrophic, and that the caudate nucleus is most affected (Harper2002).

In 1993, the HD gene, *huntingtin* (*htt*) was discovered by the ten-year collective effort of six teams in the United States and Britain, the Huntington Disease Collaborative Research Group. Soon after, several genetic models were developed including several transgenic models in *Drosophila* (Krench and Littleton 2013), nematode *C elegans*(Bates *et al.* 2006), minipigs (Baxa M *et al.* 2013), primates (Yang SH *et al.*, 2008), sheep (Jacobsen JC *et al.* 2010) and knock-in mouse models (Mangiarini *et al.* 1996; Reddy *et al.* 1998; Hodgson *et al.* 1999). Increasing evidence has converged to indicate that the normal protein functions in intracellular vesicular trafficking, endocytosis, synaptic functioning, autophagy and transcription and that the mutant *htt* has been linked to disruption of transcription, oxidative stress, autophagy, aggregate formation and several other functions in the cellular homeostasis. The mutant *htt* acquires a toxic gain of function leading to abnormal functioning but the exact pathway of cell death is still elusive.

Epidemiology of HD

Epidemiological studies have now assumed a major role in the study of various neurological disorders like Parkinson's disease, multiple sclerosis and stroke where the information is used in determining hypotheses about the societal causes, if any, of these diseases,

as well as for simply documenting their frequency and variation in the society. In case of HD, the situation is different because it is a monogenic Mendelian disorder. The route to understand the pathogenesis of the disease is principally molecular, not epidemiological. To study the frequency of the affected population it involves the careful study of complete families, rather than surveys restricted to primary cases, while those at risk are also mostly family members, rather than population at large.

Prevalence of HD in different countries

The incidence of HD is variable across the world with Japan, South Africa and Finland having very low rates of the disease among the population as depicted in Table 1. Previous estimates of 4-10 per 100,000 people in Western Hemisphere were low (Harper 2002) as recent prevalence data from UK suggests higher prevalence of adult HD at 12.3 per 100,000 based on diagnoses recorded in general practice records (Evans SJ *et al.* 2013). It is assumed that the disease spread across the globe due to migration of affected people from North-West Europe. While detailing about the prevalence of HD, one cannot leave aside the presence of sizeable population of HD patients in Venezuela. The remarkable concentration of HD patients living by the shores of Lake Maracaibo, Venezuela represents the single largest cluster derived from a single ancestor that has remained localized. The pedigree contains over 10,000 members with over 100 living subjects. The high frequency of the disorder in this region was first documented by Negrette (Okun and Thommi 2004). This particular population was recognized by Hereditary Disease Foundation to carry out a systematic study. It was easier to study the genealogy, pedigree analysis and homozygotes as several families had both the parents affected, it was a landmark project in the history of HD, which gave a new direction to HD research. The Venezuela project helped in mapping and isolation of HD gene, which led to the beginning of molecular research in HD (Wexler *et al.* 2004).

Pathophysiology of HD

The pathophysiology of HD contributed by mutant *HTT* is highly complex impacting various functional domains, as is evident from the behavioral expression, neuroanatomical topography and neurochemical profiles of affected individuals.

Behavioral symptoms

Behavioral symptoms are obvious from the impairment in motor, cognitive and psychiatric attributes. Although motor problems are the major phenotypes seen in HD patients, the psychiatric symptoms and cognitive deficits precede it and they also cause significant burden on a patient's life and lead them to complete loss of independence.

Motor abnormalities

Clinically the motor disturbances in HD is characterized by involuntary movements i.e., chorea or dystonia, all of which impair initiation, or execution of movements (Kirkwood *et al.* 2002). Motor abnormalities like bradykinesia, chorea, dystonia and oculomotor symptoms have been shown to be progressive in HD patients (Andrich *et al.* 2007). HD patients also suffer from motor problem, which is more like an inability to maintain a voluntary muscle contraction at a constant level. As a result, they cannot maintain the constant pressure during a handshake and is treated as a characteristic of HD, known as milkmaid's grip (Walker 2007). As the disease progresses, hyperkinetic movements lessen and bradykinesia and rigidity become more prominent (Novak and Tabrizi 2010).

Psychiatric and cognitive changes

Cognitive decline, with progression from early changes in speed of information processing, cognitive inflexibility, and memory retrieval to more severe and widespread abnormalities later in the disease course has been demonstrated as part of the HD phenotype by researchers (Verny *et al.* 2007). Many investigators have noted that the pattern of cognitive deficits seen in HD is similar to neurological or psychiatric conditions that disrupt functioning in the frontal lobe or basal ganglia, or conditions such as lesions in the pre-frontal cortex (Brown and Marsden 1988). Severe psychiatric and cognitive impairments have also been observed in juvenile HD patients (Riba *et al.* 2007). One of the salient features of George Huntington's description of patients suffering from the disease was "a tendency to insanity and suicide". HD patients have 4 to 6 times higher suicidal tendency than the general population (Di Maio *et al.* 1993). This rate is even higher for patients whose age is more than 50 years (Schoenfeld *et al.* 1984).

HD patients have been shown to suffer from depression, apathy and irritability when analyzed on a Unified Huntington's Disease Rating Scale (UHDRS) (Kingma *et al.* 2008). Apathy is also common, which is characterized by loss of interest and passive behavior. Working memory deficits have been found in HD patients, when event-related functional magnetic resonance imaging and a parametric verbal working memory task were used to investigate cerebral function

(Wolf *et al.* 2008). Memory acquisition and ability to concentrate are poor in HD patients due to subcortical degeneration (Zakzanis 1998). Sleep disturbance is a cause of significant distress in HD patients (Videnovic *et al.* 2009).

Anatomical features

The most striking neuropathological feature of the HD brain is the shrunken appearance of the neostriatum with gross atrophy of the caudate nucleus and putamen, with the caudate nucleus reduced to a rim of tissue. Reduction in size of the caudate is accompanied by the enlargement of ventricles. HD brains weigh less than brains of age-matched controls with the weight being reduced by 10-20 percent. Another notable feature is the loss of white matter in the subcortical zone. As a result, the brain is smaller than normal in the late stage of the disease (Vonsattel and DiFiglia 1998)

The striatum is composed of heterogeneous compartments that contain distinct neurochemicals and project to different target regions. These compartments are found in the form of patches termed as striosomes and matrix (Graybiel 1990). Striosomes consist of discrete zones and mainly contain opioid receptors, substance P, met-enkephalin and cholecystokinin. The matrix is rich in somatostatin, neuropeptide Y, nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase, acetylcholinesterase, calbindin and cytochrome oxidase. Several conflicting reports associated with the neuropathology of these two compartments in HD exist in the literature. Some describe that the total area of matrix is changed but the total area of striosomes remains the same, as determined by the staining of acetylcholinesterase or calbindin (Ferrante *et al.* 1987; Seto-Ohshima *et al.* 1988). Other studies have suggested the involvement of striosomes in the early phases of the disease (Reiner *et al.* 1988; Hedreen and Folstein 1995). The atrophied striatum is characterized by marked neuronal loss and astrogliosis, which has been used to qualify the neuropathology (Vonsattel *et al.* 1985; Myers *et al.* 1988; Heinsen *et al.* 1994). Quantitative microscopic studies have revealed that there is a relative sparing of the large striatal neurons but severe loss of medium sized striatal neurons. The majority of these medium sized striatal neurons are gamma-aminobutyric acid (GABA)-ergic in nature. As these neurons degenerate, there is a corresponding loss of the neurochemicals they contain including glutamic acid decarboxylase (GAD), Substance P, enkephalin, calcineurin, calbindin, and adenosine, together with failure of dopaminergic receptors in the striatum. According to their neurochemical differences and

differential connectivity the medium spiny neurons are of two kinds. Medium spiny neurons which express D1 dopamine receptors and Substance P project to internal segment of globus pallidus (globus pallidus interna, GPi) and substantia nigra pars compacta (SNpc), whereas those expressing D2 dopamine receptors and enkephalin project to external segment of globus pallidus (globus pallidus externa, GPe) (Gerfen 1992). One study of 17 early and middle grade HD cases found that the enkephalin containing neurons connecting to GPe were much more affected than the Substance P containing neurons projecting to GPi (Reiner *et al.* 1988). In two other studies, striatal neurons projecting to GPe showed evident loss, whereas the neurons projecting to GPi appeared relatively spared at presymptomatic or early stages of the disease (Albin *et al.* 1990; 1992).

In addition to the medium spiny neurons the striatum has various interneurons, which include the large cholinergic neurons and the sparse medium spiny neurons containing somatostatin, neuropeptide Y and NADPH diaphorase neurons. The striatal interneurons are relatively spared in HD (Ferrante *et al.* 1987; Cicchetti *et al.* 2000). In a major study on neuropathology of HD, 163 postmortem HD brains were processed and evaluated for assigning a five-point grading scale for the severity of striatal neuropathological involvement in HD with 0 indicating no abnormality and 4 signifying very severe involvement (Vonsattel *et al.* 1985). The grading was based on the histopathological features of relative neuronal loss and relative gliosis in different areas of the brain, which included the cortical, subcortical, and brain stem. Other factors like clinical records of the 163 HD cases were reviewed which included the sex, age at death and a rating of physical disability. Grade 0 was assigned to those cases in which there was substantial evidence for the diagnosis of HD, yet no gross or microscopic abnormalities that could be related to HD neuropathology. In grade 1 brains, there were no macroscopically distinguishable alterations at the level of globus pallidus (GP) and caudate nucleus-nucleus accumbens-septiputamen (CAP). However, there was moderate fibrillary astrocytosis when observed microscopically. Further, the extent of neuronal loss was evident only after cell counting and the putamen showed slight astrocytosis throughout. Grade 2 brains had atrophied caudate nucleus (CN) at the head region at CAP level, which was visible macroscopically. Microscopically, neuronal loss was observed in CAP with concomitant fibrillary astrocytosis in CN and putamen. At GP level, the CN displayed a marked neuronal loss and astrocytosis. The globus pallidus showed minimal or no changes. Grade 3 brains had a shrunken CN at CAP level. At GP level, the

CN was reduced to a thin strip. Both the putamen and globus pallidus were moderately decreased in size. Moderate neuronal loss with fibrillary astrocytosis involved the gray matter bridges between CN and putamen. Neuronal loss and gliosis is evident macroscopically in putamen. The lateral segment of the GP showed slight to moderate fibrillary astrocytosis adjacent to putamen. In grade 4 brains at the CAP level, the CN was extremely shrunken and yellow brown. The putamen was markedly atrophic with a concave medial outline. At GP level, the CN was reduced to a thin strip. The putamen was markedly reduced in size and showed widened perivascular spaces in its ventral portion. Microscopically, neuronal depletion and astrocytosis was extremely severe and diffuse throughout the CN and putamen at both levels CAP and GP. The nucleus accumbens showed slight to moderate fibrillary astrocytosis dorsally. This grading system devised by Vonsattel *et al.* (1985) has been quite useful in HD research, as it remains the single largest study of the neuropathology of post-mortem HD brains.

Although the loss of neurons from (NCP) nucleus caudatus putamen is the most conspicuous feature of HD brains, there are several reports of the involvement of other brain areas in HD neuropathology. Atrophy of the cerebral cortex has long been recognized as occurring in HD. In HD, the most affected regions in the cortex are layers III, V & VI. Previous studies have confirmed the thinning of the cerebral cortex and underlying white matter (Hedreen *et al.* 1991; Heinsen *et al.* 1994; Macdonald *et al.* 1997; Ciarmiello *et al.* 2006). Recent investigation using *in vivo* magnetic resonance imaging reveals that the behavioral deficits found in the HD patients are closely associated with discrete cortical degeneration in the brain (Rosas *et al.* 2008). Hippocampal CA3, CA4 and granule cell layer of dentate too show neuronal loss (Spargo *et al.* 1993). Neuronal loss has also been reported in ventrolateral thalamus (Dom *et al.* 1976) and in the centromedianparafascicular complex in thalamus in HD brains (Heinsen *et al.* 1996). Cerebellar atrophy has been reported in some cases of HD (Jeste *et al.* 1984; Rodda 1981) with loss of Purkinje cells in some cases (McCaughey 1961).

Microscopic examination of sections after staining them with different microglia markers also revealed reactive microglia in striatum, neocortex and internal capsule (Sapp *et al.* 2001). With the discovery of mutant *htt* and its study in transgenic models of mice helped to identify neuronal nuclear inclusion bodies in HD brains (Davies *et al.* 1997; Becher *et al.* 1998). These inclusion bodies stain positively for IC2 antibody raised against TATA binding protein with 38 or

more polyQ stretch. The inclusion bodies involve about 7% of neocortical neurons but are virtually absent in GP or cerebellum (Gutkunst *et al.* 1999).

Neurochemical alterations in HD

Neurochemical alterations may not only reflect changes in tissue pathology but also give insight into the cause of cell death in specific brain nuclei in HD.

GABA

The first neurotransmitter shown to be decreased in HD brain was GABA. Significant decrease in level of GABA and its synthetic enzyme GAD was shown in post-mortem HD brain (Perry *et al.* 1973; Bird and Iversen 1974). The striatal medium spiny neurons that are GABAergic in nature are most vulnerable neurons in HD and their demise is considered to be the neuropathological hallmark of HD (Vonsattel *et al.* 1985; DiFiglia 1990). Apart from striatum decreased levels of GABA have been found in hippocampus and cerebral cortex (Reynolds and Pearson 1987). In addition, levels of GAD were found to be reduced throughout the brain, although most markedly in the striatum and GPe (Spokes 1980). GABA receptors were decreased in striatum but unchanged in the cortex (Lloyd *et al.* 1977).

Dopamine

Dopamine levels were found to be increased (Spokes 1980) or unchanged in striatum, nucleus accumbens, and SNpc (Reynolds and Garrett 1986). Treatment of primary cultures of striatal neurons with DA caused striatal neurodegeneration with reduction in mitochondrial complex-II activity ((Benchoua *et al.* 2008). No significant alterations were found in the dopamine synthesizing enzyme tyrosine hydroxylase in post mortem HD striatum (Bird and Iversen 1977). Deficit in vesicular monoamine transporter type 2 (VMAT2) expression was found in posterior putamen in post mortem HD brains (Bohnen *et al.* 2000). *In vivo* D₂ receptor binding studies using Positron Emission Tomography (PET) with the help of the specific receptor ligand, raclopride in HD patients revealed a loss of the postsynaptic receptor in striatum and in temporal and frontal cortex (Pavese *et al.* 2003). Similar observations have been made for both D₁ and D₂ receptors in HD patient's striata using PET analysis (Turjanski *et al.* 1995).

Acetylcholine

Muscarinic cholinergic receptor expression and choline acetyltransferase enzyme activity were found to be significantly decreased in caudate nucleus of HD brain but not in the

cortex (Enna *et al.* 1976). Vesicular acetylcholine transporter (VACht) has been found to be reduced in post mortem striatal HD brain (Smith *et al.* 2006). However, large cholinergic interneurons in striatum are spared in HD.

Serotonin

Serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were found to be increased in pallidum, cortex and basal ganglia in post mortem HD brain (Reynolds and Pearson 1987; Kish *et al.* 1987).

Brain Derived Neurotrophic factor

Among different trophic factors supporting neuronal cell growth and maintenance in the striatum, BDNF has been found to be consistently decreased in HD patients and different transgenic HD models (Ferreret *et al.* 2000; Baquet ZC *et al.* 2004). Reduced BDNF induced TrkB receptor signaling has been observed in striatal cells of R6/2 transgenic mice model of HD (Nguyen KQ *et al.* 2016).

Cysteine

Cystathione γ -lyase, an enzyme important for synthesis of the amino acid cysteine has been found to be depleted in HD patients brain, striatal cells, and transgenic mouse models and cysteine supplementation in diet led to significant neuroprotection in HD models (Paul BD *et al.* 2014).

The biology of huntingtin: cellular basis of pathology

The discovery of the HD gene in 1993 made it possible to generate *in vitro* and *in vivo* genetic models in order to study the physiopathology of the mutant gene. Studies were designed to investigate the normal function of htt and its various interactions in normal and mutant forms to identify the pathways, which lead to cell death (Fig 1). The HD gene contains 67 exons and extends across 170 kb of DNA (Baxendale *et al.* 1995). The CAG repeat that is expanded on HD chromosomes lies within exon 1 and is translated into a stretch of polyQ residues. The protein HTT expressed from the HD gene has over 3000 amino acids, giving a molecular mass of around 350 kDa and is well conserved from flies to mammals (Saudou andHambert 2016). However, the amino terminal region has been extensively studied, as it contains an expandable polyQ stretch which is considered pathogenic. Several motifs have been identified including the polyglutamine and polyproline tracts close to the N-terminus and the several HEAT (Htt, Elongation factor 3, the PR65/A subunit of protein phosphatase 2A, and the lipid kinase TOR) repeats found just

downstream of the proline-rich regions C-terminal to the glutamine tract (Andrade and Bork 1995). A HEAT repeat is a degenerate of a generally 50-amino acid motif consisting of two anti-parallel α -helices forming a helical hairpin. HEAT repeat proteins generally mediate important protein-protein interactions involved in cytoplasmic and nuclear transport, microtubule dynamics, and chromosome segregation (Neuwalder *et al.* 2000). Using cross-species comparative analysis, Takano and Gusella (2002) predicted that vertebrate *htt* contains 28–36 HEAT repeats that span the entire protein. Human *htt* has rat and mouse homologues (Barnes *et al.* 1994; Schmitt 1995). It also has recognizable orthologues with the Puffer fish, *Fugu rubripes* (Baxendale *et al.* 1995) and the Zebra fish, *Danio rerio* (Karlovich *et al.* 1998).

HTT gene has two mRNA transcripts of 10336 bp and 13711 bp (Lin *et al.* 1993) and the second transcript which has an additional 3' UTR sequence of 3360 bp seems to be enriched in the brain. HTT transcripts and protein are found to be expressed at different levels throughout the humans and murine tissue (Marques Sousa and Humbert 2013). Although *htt* expression is not restricted to brain regions which preferentially degenerate in HD, as *htt* is found to be expressed in several kinds of striatal projection neurons and interneurons as well as cortex, hippocampus and cerebellum. The expression of *htt* starts in embryonic stage and its knock out is lethal on embryonic day 7.5 (Zeitlin *et al.* 1995) and persists in adulthood but the preferential toxicity in striatal cell death and the manifestation of the disease in adulthood adds a layer of complexity to the disease.

Mutant huntingtin and pathogenesis

Expansion of polyQ tract in *htt* to greater than 36Q causes the disease and it has been observed that as the number of polyQ repeats increase the younger is age of onset (Narain *et al.* 1999). It has been observed that ectopic expression of proteins containing polyQ expansion are toxic in cells, however, a loss of huntingtin function can also contribute towards the progression of the disease.

Mutant *htt* and inclusion bodies

Postmortem HD brain samples and transgenic HD mice models expressing mutant *htt* with increased polyQ repeats revealed densely stained intraneuronal inclusions (Davies *et al.* 1997). Inclusion bodies have been reported from multiple regions in the brain including striatum, cerebral cortex, cerebellum, brain stem and spinal cord. Longer polyQ tracts of *htt* have been found to have increased propensity to form aggregates of mutant *htt* with ubiquitin positive proteins (Finkbeiner S 2011). Immunostaining of inclusion bodies have revealed positive staining of

huntingtin and ubiquitin proteasome degradation related proteins (Becher *et al.* 1998). Huntingtin protein is subjected to proteolysis by a variety of proteases. There is also an increase in protease activity in the brains of patients. It is possible that the disease specific enhancement of proteolysis is an important step as it leads to the generation of small N terminal fragments that contain polyQ stretch and helps to translocate into the nucleus where they are toxic as depicted in Fig 1 (Graham *et al.* 2006). Inclusion bodies have also been reported in cytosol and neuronal processes (Gutekunst *et al.* 1999). Initial reports of inclusion bodies containing mutant htt in postmortem brain samples patients and HD mice models tempted researchers to conclude these are toxic to the host neurons. However, intense research has led to an argument about neuroprotective mechanisms of these aggregate proteins (Zuchner T and Brundin P 2008; Arrasate *et al.* 2004). A number of studies found that the localization of inclusion bodies and the vulnerability and demise of neurons in HD did not correlate well (Miller *et al.* 2010; Slow EJ *et al.* 2005). The paradigms tested to arrive at this juncture also led some researchers to pharmacologically promote IBs formation as a possible therapeutic approach for HD (Chopra *et al.* 2007). However, recent reports suggest that soluble forms of mutant htt aggregates and insoluble forms may have different level of toxicities and may regulate cell survival and death in different ways (Xi *et al.* 2016). Another recent study suggests that although soluble htt aggregates lead to apoptotic cell death but insoluble aggregates of mutant htt leads to a delayed necrotic cell death (Ramdzan *et al.* 2017). In yet another important study, it was found that soluble forms of mutant htt were severely toxic to cells by interacting with larger repertoire of proteins related to ribosome biogenesis, translation, transcription and vesicle transport as compared to the insoluble aggregates of mutant htt which had a smaller interactome which were related to quality control such as chaperons and ubiquitin-protease system (Kim *et al.* 2016).

Mutant htt and aggregate formation

Studies on mutant htt aggregation have found that its aggregation can be affected by several intrinsic factors such as the length of polyQ stretch, the amino acid residues flanking the polyQ stretch and the conformation of mutant htt. Earlier, mutant htt with increased polyQ stretches showed aggregation pattern in budding yeast (Krobitsch and Lindquist 2000) and in primary striatal culture neurons (Miller *et al.* 2010). Besides the expandable polyQ repeat, the flanking proline rich domain towards the C-terminus and the 17-amino acid peptide towards the N- terminus have been found to influence the mutant htt's capacity to aggregate. In immortalized striatal cell culture it has been observed that proline rich domain is necessary to form visible

aggregates of mutant htt (Steffan *et al.* 2004). In contrast, attaching a proline rich sequence flanking the C-terminus of the polyQ domain decreased the rate of aggregation in vitro (Bhattacharyya *et al.* 2006). Similarly, mutant htt lacking the first 17 amino acid residues at the N-terminus showed delayed aggregation in cells (Thakur *et al.* 2009). It has also been observed that although the N17 residues help the cleaved mutant htt to localize in the nucleus but it is helpful in mitigating the disease pathology in HD transgenic mice (Gu *et al.* 2016) and a HD transgenic Zebra fish (Veldman *et al.* 2015). Besides, the 17 N terminus amino acids are also secondarily modified such as dephosphorylated (Branco-Santos *et al.* 2017) and phosphorylation (DiGiovanni *et al.* 2016) which regulates its aggregation, localization and toxicity. Mutant htt has also been found to disrupt the nuclear pore complex (Fig 1), which is important for nuclear-cytoplasm transport of proteins and other molecules (Grimaet *et al.* 2017; Gasset-Rosa F *et al.* 2017). However, proteolysis of wild type *HTT* has not been reported in normal individuals. The proteolysis of wild type *HTT* may inactivate some of its normal function (El-Daher *et al.* 2015).

Post translational modifications of mutant htt

HTT is subjected to multiple post-translational modifications which include phosphorylation, acetylation, palmitoylation, ubiquitinylation and sumoylation. Most of these modifications have been studied in mutant *HTT* which could have therapeutic implications as they modify the interactions and functions of the protein. *HTT* interacts with HIP 14 and HIP14L that belong to the family of palmitoyl-acyl transferases (Yanai *et al.* 2006). *HTT* is palmitoylated at C214 and its polyQ expansion leads to a reduction in the enzymatic activity of HIP 14 and of its auto palmitoylation (Huang *et al.* 2011) leading to the enzymatic activity of HIP 14 on other substrates and therefore, regulate intracellular trafficking and synaptic localization of other neuronal proteins.

Mutant htt and autophagy

Autophagy is an important cellular process which is responsible for the removal of damaged organelles and aggregated proteins by delivering them to lysosomes for degradation. Autophagy defects have been consistently observed in HD (Steffan *et al.* 2010; Martin *et al.* 2014). High amount of autophagosome formation a reduced capacity to degrade aggregated proteins and organelles has been found in HD models (Martin *et al.* 2014). Studies also suggest that wild-type htt may play a significant role in autophagy. Reducing *HTT* expression decreased optineurin interaction in Golgi apparatus hints that *HTT* may also regulate dynamics of autophagosome

through its interaction with optineurin (del Toro *et al.* 2008). Autophagosomes in neurons under basal conditions are generated at distal axons and are retrogradely trafficked to the cell body. Silencing of htt blocks the retrograde transport of autophagosomes along the axon (Wong and Holzbaur 2014). Similar defects were observed with mutant htt, which led to inefficient clearance of mutant htt, suggesting that htt may regulate its own clearance.

In human HD and transgenic HD rodent samples mutant htt has been found to activate autophagy by sequestering and inactivating mTOR leading to an induction of autophagy (Ravikumar *et al.* 2004). The mechanistic defects in autophagy in HD are exaggerated by mutant htt. Mutant htt results in defective autophagy mediated degradation and aggregate formation, which leads to compensatory upregulation of autophagy and accumulation of mutant htt and neurotoxicity. Further due to decreased cargo movement of autophagosome to fuse with lysosomes leading to defective autophagy (Wong and Holzbaur 2014). Furthermore, expression levels of autophagy genes have been observed in HD striatum (Hodges *et al.* 2006). In the caudate nucleus of HD patients, mRNA expression of LC3A, ULK2, and LAMP2 is significantly increased whereas; PINK1, WDFY3 and FK506 binding protein 1A are significantly decreased. These findings suggest that the increased expression of LC3A and ULK2 correlate to early autophagy induction and autophagosome formation in HD (Martinez-Vicente *et al.* 2010). A decrease in PINK1 expression induces mitochondrial fragmentation and mitophagy (Kamat *et al.* 2014). HD is associated with increased mitochondrial fragmentation and inefficient incorporation of mitochondria into autophagosomes (Wong and Holzbaur 2014). Autophagy defects in HD may be a combined effect of loss of function of wildtype htt and a toxic gain of function by mutant htt.

Vesicular transport and mutant htt

HTT has been found to be involved in a variety of vesicular trafficking in neurons including synaptic precursor vesicles (Zala *et al.* 2013), autophagosomes (Wong and Holzbaur 2014), lysosomes (Liotet *et al.* 2013), BDNF containing vesicles (Gauthier *et al.* 2004) and GABA containing vesicles (Twelvetrees *et al.* 2010). It's not clear whether htt also promotes mitochondrial transport in neurons (Trushina *et al.* 2004) but it has been observed that the mutant HTT has an association with the fusion-fission machinery of the organelle (Costa *et al.* 2010; Song *et al.* 2011; Pandey *et al.* 2010). HTT is also found to interact with several proteins involved in clathrin-mediated endocytosis (Legendre-Guillemin *et al.* 2002). HTT also activated GTPase Rab11 that participates in vesicle recycling during endocytosis (Li *et al.* 2008).

Evidences suggest that *HTT* is required for ciliogenesis (Haremakiet *al.* 2015). HTT is found at the base of the cilia in neurons, photoreceptor cilia, and cilia in multiciliated cells (Keryeret *al.* 2011). Absence of HTT from mouse cells impairs the retrograde trafficking of the pericentriolar material 1 protein and the primary cilium is not formed.

Transcriptional dysregulation and mutant htt

Postmortem samples from HD brain and transgenic mouse models show transcriptional dysregulation (Valor 2015). Wildtype *htt* binds to several transcription factors including CREB binding protein (Steffan *et al.* 2000), p53 tumor suppressor, nuclear factor- κ B (Takano and Gusella 2002), PPAR- γ , vitamin D receptor, thyroid hormone receptor- α 1 (Futter *et al.* 2009). With these interactions, HTT can potentiate transcription factors which can have various outcomes (Fig 1). Transcriptional studies in rat PC12 cells expressing doxycycline induced mutant *htt* with increasing polyQ repeats were done in early stage, aggregate formed stage and late toxic stage and compared. Two major clusters of gene expression changes were observed in aggregate and later stage which were related to mitochondrial dysfunction and developmental processes related to cellular homeostasis (van Hagen *et al.* 2017). Transcriptome analysis of HD when compared to normal brains revealed wide spread aberrant alternative splicing in the diseased brains (Lin *et al.* 2016). RNA seq data from myeloid cells of HD patients revealed increases in proinflammatory cytokines in resting stage which indicates abnormal basal activation to an exaggerated immune response to a stimulus (Miller *et al.* 2016). In another RNA seq study from HD brain samples it was found that there was upregulation of developmental homeobox genes and neuroinflammatory genes (Labadorf *et al.* 2015). Multiple studies related to transcriptional dysregulation in HD samples reveal changes in inflammatory responses regulated by NF- κ B, metabolic homeostasis related to mitochondrial oxidative phosphorylation and developmental genes related to neuronal growth and cell cycle regulation.

Altered proteostasis and mutant htt

Mutant *htt* is prone to aggregate formation whose soluble and insoluble forms are neurotoxic to cells. Such misfolded forms of proteins are regulated by different proteostasis mechanisms such as chaperone mediated folding or degraded by ubiquitin mediated degradation or by lysosomal-autophagy degradation (Fig 1). However, the increasing load of chaperons found in inclusion bodies such Hsp70 and ubiquitin related protein degradation machinery or accumulation of autophagosome related target proteins lead to altered proteostasis. The expanded

polyQ fibrils can deregulate proteostasis nodes by sequestering transcription factors (Suhr *et al.* 2001) or physically obstruct neuronal extensions (Gunawardena *et al.* 2003). Moreover aging is also a risk factor for altered proteostasis (Hartl 2016). Chaperone functions are known to be compromised in HD (Kakkar *et al.* 2014).

The differential ability of neurons to handle stress of altered proteostasis could also explain the vulnerability of striatal neurons in HD. Cerebellar neurons have been observed to induce Hsp70 expression upon mutant htt expression. However, striatal neurons cannot upregulate their chaperone expression to overcome this proteostasis stress (Tagawa *et al.* 2007). It is also possible that distinct nodes of proteostasis may have unique ways of handling proteostatic stress in HD. Manipulation of different proteostatic nodes genetically or pharmacologically could be another way to handle the toxicity of mutant htt (Koyuncu *et al.* 2017)

Therapeutic interventions in HD

The cardinal features of HD like (a) movement disorder characterized initially by chorea and later by dystonia and parkinsonism, (b) progressive cognitive decline and disordered behavior, and (c) depression and agitation appear mostly during adulthood and progress relentlessly leading to functional disability. Parkinsonism is frequently observed in the rare juvenile form, slowing saccadic movements of the eye and possible cerebellar dysfunction helps in differential diagnosis. Neuropsychiatric symptoms are common and do not show increased progression with disease severity. The multifaceted phenotype of HD makes it difficult to manage the patients. Clinical trials of drugs are being conducted in every possible ways for HD therapy. Both uncontrolled and open labeled treatment reports as well as controlled trials have been carried out, where the intervention studied is compared to an active or inactive (placebo) comparator. Most of the drugs are symptomatic in nature where they are targeted to improve the clinical features of illness but do not block the progressive nature of the disease. Presently, there is no internationally recognized standard care for HD. Therefore, throughout the world the therapeutic approaches widely vary, according to the license of the respective country.

Symptomatic drugs

Most of the symptomatic drugs are directed against chorea, which is the most conspicuous feature of the disease. These drugs either deplete dopamine reserves or block the dopaminergic receptors in the brain thereby providing temporary relief to the patient. Reserpine, tetrabenazine (a short-acting reserpine analogue), phenothiazines (such as chlorpromazines) and

butyrophenones (such as haloperidol) have been used extensively for symptomatic relief in HD (Marsden 1973). In a double-blind crossover trial involving four treatments of haloperidol, haloperidol and lithium carbonate, lithium carbonate and placebo carried out for 3 weeks none of the treatments provided effective relief from choreiform movements. Psychological variables like levels of irritability, outbursts of anger and depression did appear to have some effect due to these drugs. While 3 HD patients showed some relief in haloperidol and lithium carbonate combination, the other 3 did not show any significant relief. Haloperidol itself increased the levels of depression when compared to other combinations including placebo (Leonard *et al.* 1975). In another study, 18 patients with Huntington's chorea were examined before and after treatment with 3 neuroleptic drugs, pimozide, haloperidol and tiapride. Pimozide and haloperidol gave relief from hyperkinesias but none of the drug improved motor performance significantly (Girotti *et al.* 1984). In an interesting study HD patients having gait abnormalities and choreiform movements were treated with haloperidol. Although there was a decrease in choreiform movement, yet it did not correct gait abnormalities (Koller and Trimble 1985). Long-term treatment of haloperidol is known to cause aggravated parkinsonism, tardive dyskinesia, difficulties in swallowing and dysphasia (Emerich *et al.* 1991). Tetrabenazine, an inhibitor of vesicular monoamine transporter 2 (VMAT2) leading to depletion of dopamine and other monoamines like serotonin and norepinephrine in the CNS has been used extensively in hyperkinetic disorders especially HD. In an open labeled study described by Kenney *et al.* (2007), over 400 patients suffering from hyperkinetic movement disorders including 98 HD patients were treated with tetrabenazine at the Baylor College of Medicine Parkinson's Disease Center and Movement Disorders Clinic. There was a marked improvement in patients with choreiform movements.

The Huntington Study Group (2006) recently completed a Phase III study assessing the safety, efficacy, and dose-tolerability of tetrabenazine for ameliorating chorea in patients with HD. A total of 84 patients were randomly assigned to placebo (n = 30) or tetrabenazine (n = 54) upto 100 mg/day for 12 weeks. Based on the chorea score of the UHDRS, tetrabenazine was found to significantly reduce chorea. GABAergic strategies have included muscimol, a potent GABA mimetic agonist (Shoulson *et al.* 1978). In this double-blind study, muscimol treatment did not lead to any improvement in motor and cognitive deficits in 10 HD patients. However, administration of muscimol ameliorated chorea in the most severely affected hyperkinetic patients. It also heightened dystonia, particularly in the early onset patients who had predominant parkinsonism

and dystonic features. For the treatment of depression in HD patients, standard antidepressant medication, including selective serotonin reuptake inhibitors (SSRIs) have been used. Fluoxetine, an SSRI that is widely used as an antidepressant has been shown to be effective in HD transgenic mice (Grote *et al.* 2005) but when administered in randomized, double-blind, placebo-controlled trial of this medication in depressed and nondepressed HD patients, no differences between the treatment groups were found in total functional capacity, neurological, or cognitive ratings (Como *et al.* 1997). Pridopidine, a dopamine D2 receptor antagonist was tested in three large multicenter clinical trials of HD which has been published (Lundin A *et al.* 2010; de Yebenes JG *et al.* 2011; Huntington's study group HART investigators 2011). The MermaiHD study and the HART study found that the higher dose of 90 mg/day showed improvement in motor scores and gait balance as compared to placebo but did not reach statistical significance. Presently, a large, global, multicenter, double blind phase II trial with higher dose of pridopidine –PRIDE-HD is currently under progress to assess the drug tolerability and managing motor symptoms (Reilmann 2013)

Neuroprotective Drugs

Neuroprotective therapy is targeted at the level of genetic etiology to slow down the progressive nature of the disease. Most of the drugs tested in this case have been targeted at the level of oxidative metabolism or glutamate transmission because of the metabolic decrease in activity or increased excitotoxic mechanisms in HD.

Coenzyme Q₁₀, a principle mitochondrial cofactor involved in complex-I activity has been studied as a neuroprotective agent in HD animal models (Matthews *et al.* 1998). In a multicenter, parallel group, double blind, randomized clinical trial on coenzyme Q₁₀ along with remacemide (NMDA antagonist) in early HD patients, conducted by Huntington Study Group (2001), neither remacemide nor coenzyme Q₁₀, produced significant slowing in functional decline in early HD even after 30 months. To add to these negative data, there was an increased frequency of nausea, vomiting, and dizziness with remacemide; and increased frequency of stomach upset with coenzyme Q₁₀.

Another drug that has been extensively used in HD neuroprotective therapy is creatine. Creatine is a naturally occurring compound that, through its intermediate phosphocreatine, provides a necessary cellular reserve of high energy phosphates. There is a strong evidence to suggest that a bioenergetic defect exists in HD as discussed earlier. Creatine supplementation is intended to augment cerebral reserves of phosphates and thereby reduce neuronal metabolic and

oxidative stress, and to slow down neurodegeneration. While there have been several clinical trials of creatine in HD, none have been powered to detect significant slowing of progression or improvement in clinical symptoms. Verbessem and colleagues treated 26 HD patients with 5 mg/kg creatine and 15 patients with placebo for 1 year and found no significant differences in measures of strength, neurological status, or cognitive function (Verbessem *et al.* 2003). In an interesting study Bender and colleagues used magnetic resonance spectroscopy to examine the levels of glutamate in HD patients treated with 20 g/day for 5 days, followed by 6 g/day for 8-10 weeks. They demonstrated a significant reduction in glutamate levels in the parietooccipital cortex. This is of great significance because glutamate release and excitotoxicity are enhanced by energy deficiency and are considered to play an important role in the pathogenesis of HD (Bender *et al.* 2005). However, none of the studies are informative enough to show that creatine is neuroprotective in HD.

A small scale study of fetal transplantation with a long term follow up in HD subjects was reported in 2014 (Paganini *et al.* 2014). Twenty six HD patients (10 subjects transplanted with fetal striatal tissue) were followed for median 11.2 yrs in transplanted subjects. Transplanted subjects showed a slower decline in motor and cognitive measures (Barker RA *et al.* 2013). Another small study of five transplanted and 12 control subjects followed over 3-10 yrs showed no significant benefit on clinical or cognitive features. Pathological studies have shown poor graft survival as well as aggregation of mutant Huntington in the grafted tissue (Cicchetti F *et al.* 2014; Cisbani and Cichetti 2014). Cell replacement therapy is considered to have great potential in degenerative diseases. In this context, HD makes an effective disease condition for such therapy as it is marked by striatal neurodegeneration (Precious *et al.* 2017)

Conclusions and future study

It has been almost 25 years since the discovery of the mutant gene *htt* responsible for causing Huntington's disease, but there is still no cure available for the disease. In spite of the intense efforts to discover and find a cure for the disease most of the drugs provide palliative relief. However, the research has led to a better understanding of the protein's functions in its wild type and mutant state. The multifaceted role of the normal protein and its further anomalies in its gain of functions in mutant state makes the drug targeting more challenging. The advantage of studying this monogenic Mendelian neurodegenerative disorder has been in identifying presymptomatic

biomarkers, which also be used for tracking the progression of the disease. This helps the clinicians to find more sensitive quantifiable biomarkers apart from mere behavioral phenotypes of patients which are used for drug screening and tracking the progression of the disease. Identification of peripheral markers or neurochemical agents such as BDNF, 8-OHdG and interleukins or other molecules that could also help in finding the efficacy and tracking down the neuroprotective role of drugs in clinics (Ross and Tabrizi 2011). Brain imaging is also a very reliable and quantifiable tool to measure striatal volume, cortical thickness and ventricular volume to assess the damage in brain tissue.

Although, the transgenic knock in mice models have helped us to understand the disease better but most of them do not express the disease pathology similar to humans. This has led to an increase in numbers of different mice models which correlate to different pathological phenotypes as seen in humans. Identifying protein targets of mutant *htt* interactions which lead to disease pathology could be an important strategy. With the advent of CRISPR-Cas9 technology, gene silencing of mutant *htt* in preferred areas of neurodegenerative regions could be also considered. Better animal models which exhibit pathology closer to humans would be helpful in understanding the pathology of the disease and very helpful for preclinical screening of drugs. Induced pluripotent stem cells could also be used for primary drug screening. A combination therapy of antipsychotics along with neuronal survival agent could also an effective strategy of looking at delaying the disease progression.

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Table 1. Prevalence estimates of HD in different parts of the world

Reference	Year of prevalence study	Region	Prevalence (per 100,000)
Reed and Chandler, 1958	1940	Michigan general/ black population, USA	4.1/1.5
Folstein <i>et al.</i> , 1987	1980	Maryland general/black population, USA	5.5/6.4
Wright et al 1981	1980	South Carolina general/black population, USA	4.8/0.91
Kokmen et al. 1994	1990	Minnesota, Olmsted county, USA	2
Almqvist <i>et al.</i> , 2001	1993-2000	British Columbia, Canada	0.69
Fisher and Heyden, 2014	2011	British Columbia, Canada	13.6
Shokeir 1975	-	Manitoba and Saskatchewan, Canada	8.5
Pleydell 1955	1954	Northamptonshire, UK	6.5
Harper <i>et al.</i> 1979	1971	S Wales, UK	7.61
Simpson and Johnston, 1989	1984	Grampian region of Scotland, UK,	10.0
Palo et al 1987	1986	Finland	0.5
Conneally 1984	-	Tasmania, Australia	17.4
Pridmore, 1990	1990	Tasmania, Australia	12.1
McCusker et al 2000	1996	New South Wales, Australia	6.3
Adachi and Nakashima, 1999	1997	Western Japan	0.72
Shiwach and Lindenbaum, 1990	1990	Indian Subcontinent	1.75
Chen and Lai, 2010	2007	Taiwan	0.42
Peterlin	2009	Slovenia	5.16
Morrison <i>et al.</i> 1995	1991	N Ireland	6.4
Morrison, 2010	2010	Ireland	10.6

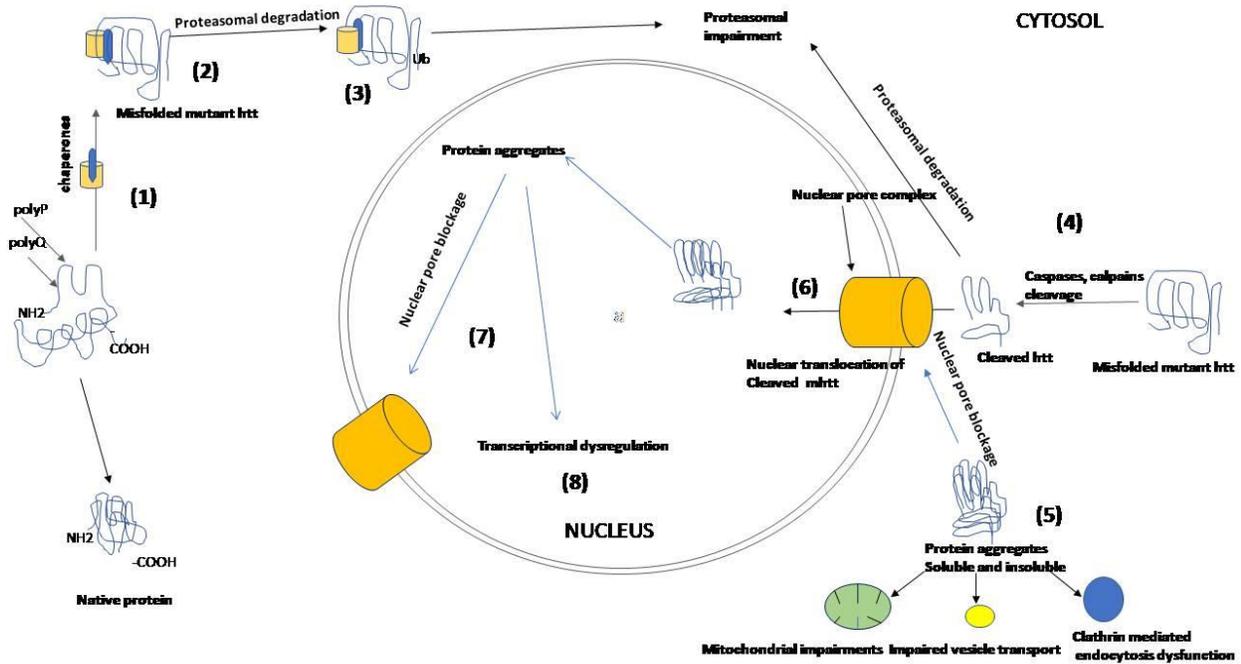


Fig 1. A diagrammatic representation of various known pathways through which mutant htt causes abnormal interactions leading to disruption of cellular homeostasis, ultimately resulting in cell demise. Mutant htt is misfolded abnormally (1) and is (2) bound to chaperones leading to (3) ubiquitination. The ubiquitinated misfolded huntingtin competes with other proteins for degradation. (4) Mutant htt is also cleaved by proteases which forms aggregates which causes (5) mitochondrial impairments, impaired autophagosome and other vesicle transport and dysfunctional endocytosis at presynaptic junctions. The cleaved N terminal mutant htt containing polyQ repeats also (6) translocates to nucleus and causes (7) nuclear pore blockage and (8) transcriptional dysregulation.