Chapter

Molecular Mechanisms of Polyglutamine Pathology and Lessons Learned from Huntington's Disease

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Abstract

Identification of polymorphic repeating units on DNA as a cause of many neurological disorders has introduced a new concept in molecular biology: Dynamic mutations. Many of the identified dynamic mutations involve expansion of trinucleotide repeats within disease genes. Nine neurodegenerative disorders are currently known to be caused by expanding CAG trinucleotide repeats. These are Huntington's Disease (HD), Dentato-Rubral Pallidoluysian Atrophy (DRPLA), Spinal and Bulbar Muscular Atrophy (SBMA), and Spinocerebellar Ataxia (SCA) Type 1, 2, 3, 6, 7 and 17. All are inherited in an autosomal dominant fashion except for SBMA, which is X-linked recessive. In all polyQ diseases, the disease mutation involves an increase in the number of CAG repeats within the coding regions of the respective genes. Since CAG triplets encode glutamine in the proteins, diseases caused by CAG repeat expansions are known as "Polyglutamine (polyQ) Diseases". PolyQ diseases share certain clinical, neuropathological and molecular findings. The most widely studied polyQ disease is HD. In HD and other polyQ diseases, conformational change in the mutant protein causes abnormal folding and proteolysis of the protein, leading to the formation of a toxic polyQ fragment, which aggregates and causes neuronal dysfunction and selective neuronal death in the brain.

Keywords: polyglutamine diseases, trinucleotide repeats, Huntington's disease, CAG expansion, neuronal death

1. PolyQ diseases

1.1 Clinical and neuropathological characteristics of PolyQ diseases

Despite a wide spectrum of clinical presentations, all polyQ diseases are characterized by late onset and progressive neurodegeneration, involving selective neuron death. Clinical symptoms generally begin in midlife, although they can also manifest earlier. In all cases, age of onset is inversely related to the CAG repeat size in respective genes. Larger CAG repeat tracts were found to be associated with earlier disease onset, severe phenotype and rapid progression [1]. The expanded repeat tracts are prone to changes in length during intergenerational transmission. This results in earlier disease onset in the later generations, known as anticipation.

The pathology of polyQ diseases involves selective neuronal degeneration mostly in the central nervous system (CNS). PolyQ disease loci, polyQ proteins and repeat sizes are shown in **Table 1**, and the clinical and neuropathological characteristics of polyQ diseases are listed in **Table 2** [2].

1.2 Genotype-phenotype correlations in polyQ diseases

CAG repeat sizes on polyQ alleles are generally categorized in three main groups, being normal, intermediate and pathogenic. Alleles with repeat numbers in the normal range are not associated with disease state. Intermediate alleles usually do not cause disease, but have a great tendency to increase in number upon vertical transmission and may cause disease in the next generation. Alleles having repeat numbers in the pathological range definitely cause the disease in a normal life span. Composition of the repeat tract is important in determining the disease state, as well as the size of the repeats. In SCA1 and SCA2, CAG repeat tracts on normal alleles are usually interrupted with other triplets, increasing the stability of DNA. However, disease alleles contain uninterrupted, pure CAG repeats. SCA1 normal alleles are almost always interrupted with one to three CAT repeats, and the disease causing expanded alleles consist of perfect CAG repeat tracts, with one reported exception of an expanded allele (58 repeats) interrupted by two CAT triplets [3]. Later onset of disease than expected in this patient suggests that the sequence composition of the repeat tract may be an important determinant in SCA1 disease manifestation. SCA2 normal alleles contain one to three CAA interruptions, with only two reported exceptions of pure 14 and 29 CAG repeats [4].

1.3 Mechanisms of polyQ pathogenesis

Although polyQ proteins are expressed widely in all cell and tissue types, each disease is characterized by selective neuron degeneration. The molecular mechanism of neurodegeneration is not clear yet, but the properties of mutant polyQ proteins provide important clues, and it is very likely that all the diseases in this group share similar mechanisms of pathogenesis. The very first effect of the repeat expansion mutation is proved to be the conformational change of the mutant protein. The new conformation of the mutant polyQ proteins was shown to confer a novel deleterious function [5].

Disease	Locus	Protein	Protein size (kDa)	CAG Repeat Ranges		
				Normal	Intermediate	Pathogenic
HD	4p16	Huntingtin	348	6–35	29–39	36–250
DRPLA	12p13	Atrophin-1	190	3–35	_	49–88
SBMA	Xq11-q12	Androgen Receptor	99	11–34	_	38–62
SCA 1	6p23	Ataxin-1	87	6–44	39–44	39–83
SCA 2	12q24.1	Ataxin-2	150	15–31	32–33	34–200
SCA 3	14q32.1	Ataxin-3	48	12–41	_	55–84
SCA 6	19p13	α_{1A}-Ca^{+2} Channel	280	4–17	_	20–33
SCA 7	3p14-21.1	Ataxin -7	96	4–35	28–35	37–306
SCA 17	6p27	TBP	42	25–42	43–46	45–63

Table 1.
PolyQ diseases.

Disease	Clinical findings	Neuropathology
HD	Involuntary choreic movements, cognitive and emotional disturbances, dementia	Cortex, basal ganglia and striatum
DRPLA	Adults: ataxia, choreathetosis, dementia Childhood: mental retardation, behavioral disturbances, myoclonus and epilepsy	Dentatorubral and pallidoluysian systems, basal ganglia, cerebellum
SBMA	Walking disability, muscle cramps, decreased deep tendon reflexes, dysarthria and dysphagia, afixation, pneumonia	Anterior horn cells in spinal cord and dorsal ganglia
SCA 1	Universal gait and limb ataxia, dysarthria, nystagmus, mild optic atrophy, hypertonia (early), hypotonia (late), decreased deep tendon reflexes, dysphagia, difficulties in breathing, extrapyramidal findings	Purkinje cells, dentate inferior olive, cranial nerve nuclei, substantia nigra, putamen, pallidum, subthalamic nucleus
SCA 2	Near universal gait and limb ataxia, dysarthria, abnormal eye movements, neuropathy, chorea, dystonia, dementia	Purkinje cells, inferior olive, pontocerebellar nuclei, substantia nigra, striatum, Clarke's column of the spinal cord, demyelination of posterior columns, cerebral cortex, spinocerebellar tracts
SCA3	Type 1: early onset, dystonia and rigidity Type 2: cerebellar and pyramidal signs Type 3: late onset, peripheral neuropathy, cerebellar signs Type 4 : parkinsonism	Globus pallidus, subthalamic nucleus, substantia nigra, dentate nucleus, pontine and cranial nerve nuclei, spinal neurons, peripheral neuropathy
SCA 6	Ophtalmoplegia, spasticity, peripheral neuropathy, dysphagia, parkinsonism, balance problems	Loss of Purkinje and granular cells, neuron death in dentate nucleus and inferior olive, atrophy in brain stem
SCA 7	Visual loss, gait and limb ataxia, dysarthria, widespread pyramidal findings, decreased vibration sense, dysphagia, hearing impairment	Retina, cerebellar Purkinje and granule cells, dentate, inferior olive, subthalamic nucleus and spinal motor neurons
SCA 17	Gait and limb ataxia, dementia, parkinsonism, chorea or dystonia	Small neurons in caudate nucleus and putamen, Purkinje cells, thalamus, frontal and temporal cortex

Table 2.
Clinical and neuropathological findings in PolyQ diseases.

Mutant polyQ proteins are cleaved by proteases, aggregate, and form inclusions in the cells. The polyQ aggregates are ubiquitinated and recruite many proteins to the aggregates, like transcription factors (TFs), chaperons, and proteasomes. The exact protein context could influence the differences in areas of neurodegeneration. Mutant proteins may lead to cell death by causing changes in protein interactions, transcriptional dysregulation and dysfunction of the ubiquitin proteasome system (UPP). On the other hand, it is also possible that partial loss of the normal functions of proteins may be contributing to disease pathogenesis [6]. So, gain and loss of function mechanisms could have convergent effects in polyQ-mediated neurotoxicity.

2. Huntington's disease

2.1 Epidemiology and clinical correlates

The first definitive description of HD was presented in 1872 by Dr. George Huntington, in his article titled "On Chorea" [7]. This article pinpoints the important

essential features of the disease; its hereditary course and its manifestation in adult life. This first full description of the disease awakened the HD research, and studies concerning the origin and prevalence of the disease have started. It has been cleared that the gene originated in Northern Europe, with a prevalence of 5–10 affected people per 100 000 among individuals of European descent. Apart from Finland, there is a uniform prevalence of HD throughout Europe. The prevalence of HD in Finland is lower than that of other Northern European countries, which can be partly explained by the lower frequency of htt haplogroup A on chromosome 4 among the Finnish population.

HD is a chronic and progressive neurodegenerative disorder of the CNS, inherited in an autosomal dominant manner. The characteristic clinical features include motor, cognitive and behavioural abnormalities. HD is regarded as an adult-onset disease since the first signs appear in the third to fifth decade of life. However, the age at onset (AO) follows a normal distribution, showing a peak around the fourth decade, with some patients manifesting the disease before 20 (Juvenile HD, 5–10 per cent of HD cases) or after 60 years of age (~20 per cent of HD cases). The illness lasts for about 15–20 years. The clinical course of HD involve three basic abnormalities: Motor dysfunction, behavioural disturbances and cognitive decline. Motor abnormalities involve involuntary movements in the extremities and progress into jerky movements. The severity of involuntary movements, known as chorea, increases progressively during the initial years of the illness, and is later replaced by bradykinesia and rigidity. In the disease course, patients also develop dysarthria, dysphagia, balance problems, and incoordination. Later on rigidity dominates, which may leave the patient bed-bound or confined to a wheelchair. In juvenile HD (JHD) patients, rigidity is more common than chorea. Behavioural abnormalities usually precede the motor symptoms, including depression, anxiety, and changes in personality, however they are usually unnoticed. Suicide, the third most common cause of death among HD patients, is more common than in the general population. Through the late stages of HD, patients develop dementia, characterized by inefficient use of memory and impairment of executive functions [8].

2.2 Neuropathology of HD

The neuropathology of HD is restricted to the brain, selective loss of neurons within the striatum being the predominant hallmark. Medium sized, spiny GABAergic striatal output neurons are lost up to 80 per cent. Neuronal loss in HD patients begins early in life, before the manifestation of motor symptoms. At the time of motor onset of disease, cortical grey matter, subcortical white matter, and about 30 per cent of caudate neurons are already lost (**Figure 1**). These all account for loss of brain weight by 20 to 30 per cent less than normal [9]. The neuronal intranuclear inclusions (NII) found in the brain are accepted as a neuropathological marker of HD. Huntingtin inclusions were shown in the cortex and striatum of transgenic HD mice, and postmortem HD brains [10]. Gamma Amino Butyric Acid (GABA), acetylcholine and dopamine neurotransmitters were found to be decreased in the diseased basal ganglia. Also glutamic acid decarboxylase (GAD) levels, the enzyme involved in GABA biosynthesis, is found to be reduced in the caudate, putamen and globus pallidus.

2.3 Molecular genetics of HD

2.3.1 The HD gene structure

In 1993, human HD gene was localized to chromosome 4p16.3 by positional cloning approach [11]. The HD gene, called IT-15, consists of 67 exons spanning 180 kb of DNA (**Figure 1**). The $(CAG)_n$ repeat is located in Exon 1, 17 codons downstream the ATG start codon, and encodes a highly polymorphic and unstable segment of

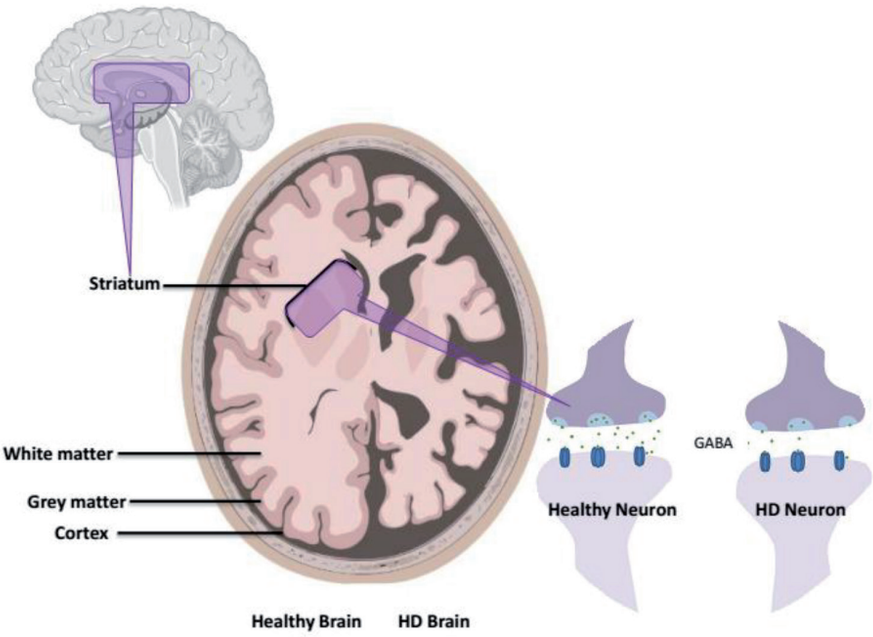

Figure 1.
Neuropathological changes in the HD brain.

glutamines. The repeat tract varies between 6–35 CAGs in healthy individuals and 36–250 CAGs in HD patients. Adjacent to $(CAG)_n$ repeats, there is a stretch of proline encoding CCG repeats, which is slightly polymorphic (6–12 repeats) and stably transmitted [12]. Most healthy chromosomes and the majority of mutant chromosomes contain seven CCG repeats. In Exon 58, there is a rare codon-loss (GAG, glutamate) polymorphism (Δ2642), which occurs in 5% of healthy and 24–38% of HD chromosomes [13]. The human HD gene is highly conserved across a wide range of species like mouse, rat, fugu, zebrafish, and pig [3, 14, 15].

2.3.2 The HD mutation

The mutation causing HD is the expansion of the CAG repeat tract in the first exon of the HD gene [11]. Healthy chromosomes contain 6–35 CAGs, and HD chromosomes possess 36–250 units [16]. The CAG repeats in the HD gene are highly unstable, resulting in expansions or contractions upon transmission to the next generation. The CAG repeat tract was shown to be unstable in 80% of the transmissions. The size and direction of the instability depend on the sex of the affected parent. In maternal transmissions, nearly equal numbers of expansions and contractions are seen, and shifts range from one to three repeats in size. In contrast, paternal transmissions are more frequently expansions. In addition to meiotic instability, a modest degree of instability was shown within and between somatic tissues of HD patients. In the brain, instability was observed in the caudate and putamen; in non-CNS tissues, most instability was observed in the liver and kidney. Three general mechanisms can be proposed to account for repeat instability: Slippage during DNA replication, misalignment with subsequent excision repair, and unequal crossover and recombination. Slippage-mediated length change during DNA replication better explains the instability in repeat size in HD.

2.3.3 Genotype-phenotype relations

There is a strong inverse correlation between age of onset (AO) and expanded CAG repeat length. The length of the expanded CAG repeat accounts for about 70%

of the variation in AO, however, repeat size alone should not be used to predict AO [16]. The second modifying factor of AO is the sex-of-parent effect, which accounts for 2–5% of the variation. Juvenile HD patients more likely inherit the disease from their fathers, and patients with late AO more frequently inherit the disease from their affected mothers [16]. The remaining variation in AO may partly be explained by environmental effects, however, studies suggest a strong genetic component, which implies that there are other genes that modify AO in HD. New mutations, resulting in de novo disease presentation have been described in HD. Higher normal repeats may expand into the pathological range and may cause disease in the next generation, leading to a new mutation in the family.

2.3.4 Huntingtin mRNA and protein

IT-15 gene is expressed ubiquitously in all human tissues in the form of two major messenger RNA (mRNA) transcripts of 13.6 kb and 10.3 kb in length, which differ in the size of the 3' UTR due to differential polyadenylation [17]. HD mRNA is expressed in both neural and non-neural tissues with high levels of expression in brain and testes [18].

The HD gene encodes a protein of 3144 amino acids with a molecular mass of 348 kDa, termed huntingtin (htt). The polyQ tract starts at residue 18 and is followed by a stretch of prolines. Protein studies also indicate ubiquitous expression of htt in various cells and tissues throughout the development and in the adult. In HD patients, normal and mutant huntingtin have a similar distribution and expression pattern [17, 18].

Huntingtin has no structural homology with other proteins, which makes the determination of its normal function difficult. The high degree of conservation across species suggests that the normal function of htt is essential. Studies of mice with targeted mutations that reduce the levels of htt to zero to 50 per cent reveal that htt also plays a critical role in embryogenesis, during brain development. Reduced levels of htt lead to perinatal lethality and abnormalities in the head region, and significantly reduced levels produce a more severe phenotype, including abnormalities in skin, placement of the ear, and excencephaly. In addition, conditional knock-out mice develop a neurodegenerative disease [19]. HD cell models revealed that the wild type (wt) protein partially protects cells from mutant htt. Htt may also be important for cell survival through growth factor (GF) stimulation, as brain-derived neurotrophic factor-rescued cells expressing mutant htt [20]. In the neuron, htt is found throughout the cell body, and in axons, dendrites, and perikarya. Its association with microtubules and vesicles [21] has suggested a role in intracellular trafficking or neurotransmission, and retrograde transport. Association of htt with the cytoskeleton and cytosolic membrane suggests a role in normal cytoskeletal function [22]. Htt is also found in cytoplasmic inclusions in Alzheimer's Disease (AD) and Parkinson's Disease, which are mostly made up of cytoskeletal proteins. The presence of htt also in the nucleus could indicate that it may be involved in nuclear processes such as transcription, replication, RNA splicing, mRNA transport, and nuclear organization. Huntingtin has no known homologies to any other protein, but it contains a few known sequence motifs with defined functions. Several proteins that interact directly with htt may give clues in understanding its possible functions. Based on their known functions, htt-interacting proteins can be grouped as proteins that are involved in gene transcription, intracellular signaling, trafficking, endocytosis or metabolism [23]. Analysis of the htt-interactors reveal that htt might function as a scaffold, controlling the proteins for signaling processes and intracellular transport [24].

2.4 Molecular pathology in HD

The general flow of the molecular pathology in HD is summarized in **Figure 2**. It starts with gain of toxic function together with loss of normal htt upon conformational change of the protein. The mutant protein aggregates and becomes more prone to proteolysis and the toxic htt fragment may interfere with transcription, leading to neuronal dysfuntion and selective neuronal death.

2.4.1 Gain and loss of function of Huntingtin

The general flow of the molecular pathology in HD is summarized in **Figure 2**. The molecular mechanism underlying polyQ pathogenesis has first been explained by toxic gain of function (GOF) of the mutant proteins. However, later findings strongly addressed loss of function of the normal proteins as a contributor to the disease process. In a mouse model, 146 CAG repeats were inserted into the HPRT gene, which is not involved in any CAG repeat disorder, in order to prove the gain of function mechanism. This mutant mice produced a polyQ expanded form of the HPRT protein and developed a late-onset neurological phenotype that resulted in death [5]. So, it is widely accepted that the polyQ mutations act by introducing a novel deleterious function to the mutant proteins. In humans, heterozygous inactivation of htt by disrupting the HD gene does not result in HD phenotype [25]. In addition, mice that have only one functioning Hdh gene do not show any features of the disease [26]. Although an expanded CAG repeat could play a major role in the disease, the possibility of a dysfunction of the wt protein in HD cannot be ruled out. These findings suggest that the toxic effect of mutant htt might lie in sequestration of normal htt or of its functions [27].

2.4.2 Conformational change of the mutant Huntingtin

The baseline of the pathogenic mechanism causing HD appears to be the unusual conformation adopted by the mutant htt protein as a result of the expanded

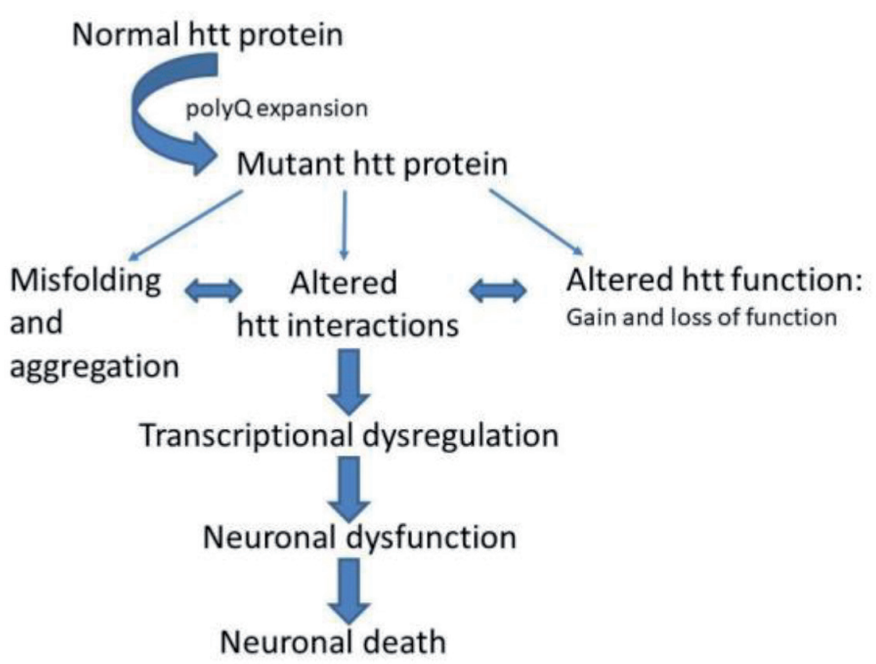

Figure 2.
Molecular pathology of HD.

polyQ segment. In a short protein fragment, the expanded polyQ tract facilitates aggregation, via conversion to an insoluble amyloid sheet structure [27]. Such a difference in the protein structure may lead to a change in the usual interactions of the protein and also may lead to abnormal interactions with other cellular factors. In addition, the mutant protein with an expanded polyQ tract may have effects on the function of the wild type protein. Two non-mutually exclusive mechanisms have been proposed to explain the aberrant conformation and subsequent aggregation of the mutant htt: Polar zipper and transglutaminase hypotheses. The polar zipper model states that the normal protein conformation is destabilized due to the presence of the expanded polyQ tract, and insolubleβ -pleated sheets may form and aggregate, by linkingβ -strands together via hydrogen bonding, forming "polar zipper" structures [28]. The transglutaminase hypothesis states that, mutant htt aggregation could be a result of the transglutaminase activity, which are normally involved in crosslinking of glutamine residues in different proteins. Huntingtin is a substrate of transglutaminase in vitro, and the rate of the reaction increases with the length of the polyQ tract [29]. According to these results, it can be hypothesized that, expanded polyQ stretch may result in increased crosslinking between mutant htt and other proteins, including itself, which may lead to aggregation.

2.4.3 Mutant protein aggregation

Transgenic mouse models of HD, expressing Exon 1 of the human HD gene with various CAG repeats numbers under the control of the human htt promoter, were established. These models served as a major step towards understanding the molecular pathology of HD. Mice expressing 18 CAG repeats developed normally and remained healthy. By contrast, mice that expressed 113 to 156 CAG repeats demonstrated progressive neurological symptoms and developed intraneuronal aggregates [21, 30]. Similar aggregates have been identified in post-mortem human cortical and striatal neurons and dystrophic neurites of HD brains [10]. Although NII occur in areas of pathology, their distribution does not correlate with the regions of neurodegeneration. In HD brains, inclusions contain truncated fragments of the mutant htt, which are recognized only by antibodies to the N-terminal region of the htt. The intranuclear inclusions are spherical or elliptical, larger than the nucleoli, and are not isolated with a membrane. Electron microscopy reveals that the inclusions contain a mixture of granules, filaments and fibrils. Intranuclear aggregates are accepted as a common pathological marker for polyQ diseases, since similar structures are seen in all polyQ diseased brains. Since the inclusions are not isolated with a membrane, they can be regarded as membrane-less compartments or organelles. The membrane-less structures, in general, are important for various essential functions in both the cytoplasm and the nucleus. They are formed through a phase separation process of RNA and protein molecules and are known to be responsive to the changes in the cellular environment. These structures are also dynamic, such that they can sequester or release proteins and RNA molecules, thereby affecting cellular stress response and neurodegenerative processes [31]. In this context, nuclear inclusions in HD should not be regarded as sole aggregates of mutant htt, but rather as a potential sink organelle for various RNA and proteins which possibly change the interactions of mutant htt and having deterministic roles in the fate of neurons with these inclusions and the neurodegenerative process in general.

Although accepted as a main pathological marker, the importance and function of the inclusions in cell culture models and HD brains are still not clear. Inclusions can be the primary cause of neuron loss, they can be beneficial by isolating the mutant protein from the rest of the cell, or just epiphenomenon. Since they appear

before the disease symptoms in the transgenic mice, a causal role has been suggested [30]. In addition, inclusions well correlate with the size of the CAG repeats and susceptibility to cell death [32, 33]. Reduction of inclusions using heat shock protein HDJ-1 decreases cell death *in vitro* [34].

2.4.4 Proteolysis

Proteolysis of htt by caspases and calpains has been shown in several studies using cell culture, animal models and post-mortem analyses [35]. The toxic fragment hypothesis suggests that, the pathological mechanism of HD may rely on the production of a toxic fragment containing the polyQ tract. The basal caspase activity may be sufficient to generate small amounts of cleavage products. If this cleavage products are toxic to neurons, then accumulation would put further stress to the cell, resulting in additional caspase activation in a positive feedback loop, and eventual cell death [36]. It is possible that over the life span of the affected individual, sufficient amounts of toxic polyQ fragments may be produced from this basal activity, pushing the balance toward cell death and neurodegeneration. The model for the role of caspases suggests two major stages. First, truncated htt fragments containing the expanded polyQ tract were produced by caspases (e.g. caspase-3, caspase-6), which can be considered as a rate-limiting step. Then, additional caspase activity (e.g. as caspase-8) can be provoked that may activate the caspases (e.g. caspase-3) further, which may eventually result in cell death.

2.4.5 Role of chaperones and proteasomes

Proteins with long polyQ tracts have altered conformation and are usually misfolded. Chaperone proteins help in the folding of proteins. Several heat shock proteins (HSP) function as modulators of protein folding, thereby prevent misfolding and aggregation [37]. It was shown that HSP-40 and HSP-70 are sequestered in htt Exon 1 aggregates in cell models [38]. It is possible that redistribution into aggregates could deplete chaperones in the cells, preventing them to perform their functions.

Mutant polyQ protein aggregates were found to be ubiquitinated and associated with the proteasome apparatus. Cells tag misfolded proteins with ubiquitin, which directs them to proteasomes for degradation. In their abnormal conformations, mutant polyQ proteins might have a restricted entry into the proteolytic chamber or they might be incompletely degraded, causing jamming of the proteasomes. The depletion of proteasomal activity might lead to a build-up of many proteins normally cleared by proteasomes. Degradation of short-lived proteins is the major function of the proteasomes. The concentrations of some of these proteins, for example transcription factos, should be under strict control, since they are critical regulators of cellular homeostasis. If the proteasomal activity is impaired, the levels of short-lived proteins will rise abnormally, leading to cellular toxicity [39, 40].

2.4.6 Aggregates, nuclear localization and toxicity

Subcellular localization of the mutant polyQ proteins may be a more important determinant of toxicity, rather than the formation of aggregates. Although there is cytoplasmic pathology, many studies draw attention to the nucleus as the major site of pathogenesis. Transgenic mice, expressing expanded polyQ proteins with mutant nuclear localization signal (NLS) do not show disease pathology, and NLS-inserted polyQ proteins cause more toxicity in the cells. In addition, htt, being a cytoplasmic protein, was found in the nucleus when mutated. These findings support the

hypothesis that nuclear localization of mutant polyQ proteins have an important role in disease pathogenesis [41]. Studies questioning aggregate toxicity and nuclear localization resulted in very important findings. When Q20 and Q42 peptides were subjected to cold shock, they formed fibrillar aggregates in the cells. When NLS was added to these peptides, aggregates entered into the nucleus, and both normal and mutated peptide aggregates resulted in cell death. However, cytoplasmic aggregates without NLS did not cause toxicity. Sixty five per cent of the cells with nuclear Q42 aggregates exhibited cell death in 24 hours. According to these findings, subcellular localization of the aggregates, rather than the length of the polyQ stretch seems to be more important. When NLS is added to amyloid fibril forming bacterial CspB-1 cold shock protein, aggregates are formed in the nucleus, but are not toxic. This proves that the polyQ tract itself is responsible for aggregate toxicity. Aggregate formation requires a critical polyQ concentration, and this can explain the late disease onset and the negative correlation between the repeat length and AO. Proteins with polyQ repeats in the normal range are also shown to form fibrillar aggregates *in vitro*. However, proteins with normal numbers of repeats require a higher threshold concentration for protein aggregation, and the aggregate formation process is very slow, which makes this event impossible in a normal life span [33, 34, 41].

2.4.7 Transcriptional dysregulation

Cleavage and subsequent nuclear localization of polyQ proteins may result in changes in nuclear functions, like nuclear protein turnover, transcription, and RNA processing, through interactions with various nuclear factors. The most widely studied mechanism among them is the transcriptional dysregulation. One hypothesis concerning transcriptional dysregulation is that polyQ proteins might normally interact with proteins in transcription complexes, and interactions might become aberrant when the polyQ is expanded [42]. Furthermore, mutant polyQ proteins can sequester other proteins containing polyQ stretches, like several transcription factors. Therefore, polyQ toxicity may arise from decreased availability of some transcription factors, resulting in abnormalities in the regulation of transcription. Htt was found to interact with proteins involved in transcriptional regulation, including transcriptional coactivators and corepressors. Mutant htt may be toxic through binding and depleting some of these factors. The most important TFs that are thought to be involved in HD pathogenesis are, CRE-binding protein (CREBP), p53, C-terminal binding protein (CtBP), TAFII 130 and Sp1 [43, 44].

2.4.8 Neuronal dysfunction and selective death in HD

The early disease process involves neuronal dysfunction rather than cell death, and in late stages apoptotic type of cell death occurs. Biochemical and immunohistochemical studies show N-terminal htt fragments in brain tissues from affected HD patients and not from controls. Also, increased levels of DNA strand breaks in HD patient brains imply an apoptotic mechanism [10]. Although the mutated proteins are expressed in many cell types [18], a specific pattern of neuronal degeneration occurs in each polyQ disease [45]. The selective vulnerability of striatal and cortical neurons in HD can not be explained by the level of htt expression, since other regions of the CNS express htt at the same level, but are not affected in the disease. Interactions of the polyQ containing proteins in specific cell types, sequestration of some tissue-specific transcription factors, or somatic increases in mutation size in the affected brain regions may provide an explanation. It is also possible that mutant proteins undergo cell type-specific proteolysis. Tissue-specific proteolysis can occur in two ways: Either the mutant proteins are processed with tissue specific

proteases, or the mutant protein in its new conformation becomes susceptible to different proteases. In fact, tissue-specific proteolysis has been demonstrated in one study, in which proteolysis specific to striatum and cortex was shown, without any differences between healthy and HD brain [46]. No striatum specific htt interactor has been identified yet in order to suggest a tissue-specific interaction of htt, but striatum-specific transcriptional regulators can be more sensitive to htt dysfunction, causing transcriptional dysregulation. On the other hand, N-terminal htt fragments accumulate in striatal neurons and their axonal processes in HD transgenic mice. This finding supports the idea that mutant htt is proteolysed specifically and aggregates selectively in striatum [6].

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Conflict of interest

I have no conflict of interests.

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