CHAPTER - II

REVIEW OF LITERATURE

2.1 Polyglutamine Diseases

2.2 Huntington’s Disease (HD)

Symptoms of Huntington’s disease

2.3 Prevalence of HD

2.4 Mechanism of Neurodegeneration

2.5 Mutant Huntingtin Protein and Intracellular Abnormalities

2.6 Genetic Testing for Huntington’s disease

2.7 Treatments

2.8 Structural Aspects of Polyglutamine

2.8.1 Spectroscopic studies

a) Circular Dichroism

b) Nuclear Magnetic Resonance Spectroscopic Investigations

2.8.2 X-ray Diffraction

2.8.3 Biological model studies

2.8.4 Polyglutamine aggregation, fibrilisation and toxicity

2.8.5 Simulation studies on polyglutamine

2.8.6 Therapeutic approaches to inhibit aggregation
REVIEW OF LITERATURE

Neurodegenerative diseases are prevalent and becoming significant, due to increasing cause of morbidity and mortality. Nearly all major neurodegenerative diseases occurred may be due to accumulation of soluble proteins to form insoluble aggregates in the central nervous system (CNS) i.e. formation of amyloid like structures (Daniel and colleagues, 2006) which are hallmark of many protein misfolding diseases like Alzheimer, Parkinson’s, Fragile X syndrome, Huntington’s, prion and many others are developing expansions in the tri-nucleotide (TNR) repeats in the respective genes. The TNR results in the congenital malformations syndrome in the individuals with these types of inherited mutations. There are number of genetic diseases known which are the consequence of expansions in the TNRs in the CAG, CCG, GCG etc. codons encoding and polyA amino acids stretches in the respective proteins which causes structural deformity and hence occurrence of the diseases. There are nine polyalanine repeat expansion disorders reported which are mitotically and meiotically stable on contrary to polyglutamine (polyQ) repeat expansion like Huntington’s chorea which is not stable in the successive generations.

On the basis of the distribution analysis of repeat-containing proteins, Faux et al., (2005) observed that 73% of the homopeptide repeats are closely located to the N or C-terminal of a structured domain. Among the disease-related polyQ proteins, about nine neurodegenerative disorders polyQ tracts located in the unstructured region is given in table 1.

2.1 POLYGLUTAMINE DISEASES

Polyglutamine (Andrew and Hayden, 1995) proteins represent unique class of misfolding neurodegenerative disorders caused by unstable cytosine-adenine-guanine (CAG) trinucleotide repeats expansion at the specific gene position of the different chromosomes in the human cell. Over the past two decade, the focus has been on to identity the mechanism by which the expanded repeats renders a protein toxic to the subset of vulnerable neurons.

In the sub family of nucleotide repeat disorders and polyalanine diseases are unique and occur with tri-nucleotide repeat expansion, and are associated with cytoplasmic or nucleoplasmic misfolded protein inclusions having a number of distinguishable features among them.
### Table 1 The polyglutamine family of neurodegenerative diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene locus</th>
<th>Pattern of inheritance</th>
<th>Protein</th>
<th>Subcellular Location</th>
<th>Pathogenic length threshold</th>
<th>PolyQ location</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD</td>
<td>4p16.3</td>
<td>AD</td>
<td>htt *</td>
<td>Cytoplasmic and nuclear</td>
<td>36–121</td>
<td>3144 aa</td>
</tr>
<tr>
<td>SBMA</td>
<td>Xq13–21</td>
<td>X-linked recessive</td>
<td>Androgen receptor (AR)</td>
<td>Nuclear and Cytoplasmic</td>
<td>38–62</td>
<td>919 aa</td>
</tr>
<tr>
<td>DRPLA</td>
<td>12p13.31</td>
<td>AD</td>
<td>Atrophin-1*</td>
<td>Cytoplasmic</td>
<td>49–88</td>
<td>1195 aa</td>
</tr>
<tr>
<td>SCA1</td>
<td>6p23</td>
<td>AD</td>
<td>Ataxin-1*</td>
<td>Nuclear (neurons)</td>
<td>39–82</td>
<td>815 aa</td>
</tr>
<tr>
<td>SCA2</td>
<td>12q24.1</td>
<td>AD</td>
<td>Ataxin-2*</td>
<td>Cytoplasmic</td>
<td>36–63</td>
<td>1313 aa</td>
</tr>
<tr>
<td>SCA3</td>
<td>14q32.1</td>
<td>AD</td>
<td>Ataxin-3</td>
<td>Cytoplasmic</td>
<td>55–84</td>
<td>354 aa</td>
</tr>
<tr>
<td>(MJ D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCA6</td>
<td>19p13</td>
<td>AD</td>
<td>Ataxin-6*</td>
<td>Cell Membrane</td>
<td>21–33</td>
<td>2505 aa</td>
</tr>
<tr>
<td>SCA7</td>
<td>3p12–13</td>
<td>AD</td>
<td>Ataxin-7*</td>
<td>Nuclear</td>
<td>37–130</td>
<td>339 aa</td>
</tr>
<tr>
<td>SCA17</td>
<td></td>
<td></td>
<td>TATA-box binding protein (TBT)</td>
<td></td>
<td>47 – 63</td>
<td>892 aa</td>
</tr>
</tbody>
</table>

*The function of this protein is not clear*
The diseases include Huntington Disease (HD), the family of Spino Cerebellar Ataxias (SCAs) comprising the disease named SCA1, SCA2, SCA3, SCA6, SCA7 (Zoghbi and Orr, 2000), SCA17 (Oda et al., 2003), Dentatorubralpallidoluysian Atrophy (DRPLA) (Gatchel and Zoghbi, 2005) and Spinobulbar muscular atrophy (SBMA). These diseases are characterized by progressive motor and cognitive degeneration caused by expansion CAG trinucleotide in specific neuronal region cerebral cortex, ganglia, brain stem, spinal cord and cerebellum that ultimately lead to death (Ramazzotti et al. 2012).

The neurodegenerative trinucleotide repeat diseases broadly fall into two distinct classes (Cummings and Zoghbi, 2000); (i) the polyQ repeat disorders, in which the expanded trinucleotide (CAG) repeat is translated into an expanded polyQ tract, which results in formation of protein aggregations within the cell, (ii) the trinucleotide repeat is present in an untranslated region of a gene, having more varied molecular mechanisms including gene repression.

Though all the neurodegenerative diseases are caused by the CAG trinucleotide repeat in different number of expansion, yet the patterns of neuronal loss, symptoms and their clinical features vary significantly. The molecular basis for evolution and origination of such diseases is still unclear and no therapeutic strategies for these intractable disorders are available. The features of the mutant protein causes selective neurodegeneration within various regions of the Central Nervous Systems (CNS) by influencing various factors such as protein-protein interactions, protein folding (protein misfolding), endogenous functions of individual and specific disease proteins, subcellular localizations, formation of inclusions, environment conditions. At the context of the CAG repeats, the expanded polyQ protein undergoes transformation from monomers to oligomers and inclusions which may ultimately lead to toxicity. The expansion does not exactly explain the selective vulnerability of the specific neurons in each disease but share a number of characteristics. It is apparent from table 1 that the minimum length for the onset of neurodegenerative disease varies with the type of disease and the maximum length for the onset of SCA3 is 55 and for SCA6 is 21. The minimum length for onset of Huntington disease is 36 (Laura Mangiarini et al., 1996).
The mechanisms for the neurodegeneration in the CAG repeat diseases, including Spinal and Bulbar Muscular Atrophy (SBMA), Huntington’s disease (HD), Dentatorubral and Pallidoluysian Atrophy (DRPLA), and Spino-Cerebellar Ataxia (SCA) varies considerably. Proposed mechanisms have included activation of caspase or other triggers of apoptosis, mitochondrial or metabolic toxicity, and interference with gene transcription (Ross, 2002).

The molecular and biochemical methods are important tools for elucidating the structural characteristics, conformation and aggregation to decipher the steps involved in the mechanisms of diseases. A little attention has been paid on the characterization of conformation and aggregation. Therefore, a systematic study is necessary to elucidate the conformation of the varying chain length and their aggregation responsible for Huntington disease. Water is a universal solvent for biological systems. Molecular dynamics simulations may provide an answer at the atomistic level. The effect of environment on the conformation and aggregation behaviour can be deciphered by simulations studies in different environments i.e. in vacuum and in water. Graphical representation of the simulated molecules can provide explanations for the adopted conformations and aggregation in terms of interactions. Molecular graphics representation can also provide an explanation for how molecules aggregate to form fibril and this may provide an explanation on the toxicity of the Huntington’s disease.

2.2 HUNTINGTON’S DISEASE

HD is an autosomal dominant neurodegenerative disorder associated with progressive degeneration of neurons in certain regions of the brain and the presence of astrocytes that accumulate due to destruction of nearby neurons (gliosis). These neurodegenerative changes primarily occur within the caudate nuclei and the putamen, substructures of the basal ganglia that are collectively known as the striatum (The basal ganglia consist of specialized nerve cell clusters deep within the brain that organize motor behaviour. Major substructures of the basal ganglia include the caudate nuclei, the putamen, and the globus pallidus as well as other cell groups). HD is also characterized by associated neuronal degeneration within the temporal and frontal lobes of...
the cerebral cortex. This part of the brain is responsible for integrating higher mental functioning, movements, and sensations.

**Symptoms of Huntington’s disease**

The signs of HD are progressive and the symptoms start gradually and proceeded in a phased manner of mild psychiatric and behavioral abnormalities. The HD affected patients exhibits nonspecific activities in personalities and mood, such as irritability, impulsiveness, social withdrawal, apathy, which are sometimes hard to distinguish from their normal behaviour (Boll and colleagues, 1974 Bylsma and colleagues, 1993). It has been observed that the most prominent cognitive impairments in HD involve the “execution of functions” such as abilities of organization, regulation, and perception (Albert and Rosenberg colleagues, 1981 and 1985; Bamford et al., 1995). The HD patients also suffer from weight loss for an unknown reason. Increased muscle activities, reduced capacity to swallow, loss of appetite, and an increased metabolic have been suggested as possible explanations. Metabolic and/or endocrine dysfunction occurs (Sanberg and colleagues, 1981 and Pratley et al., 2000) due to weight loss in HD patients and the eventual cause of death is usually aspiration pneumonia and other complications of immobility.

The degenerative changes in HD primarily affect certain nerve cells of the striatum which are named for their size and appearance and project into the globus pallidus and substantia nigra. These highly specialized “spiny” neurons secrete gamma-aminobutyric acid (GABA), a neurotransmitter that inhibits the release of neurotransmitters from other nerve cells. One school of thought believes that selective loss of these specialized cells results in decreased inhibition (i.e. increased activity) of the thalamus. Therefore, the thalamus increases its output to certain regions of the brain’s cerebral cortex. This may lead to the disorganized, excessive (hyperkinetic) movement patterns of chorea.
The HD gene (HTT) encodes a protein named huntingtin (htt). The expansion of trinucleotide CAG repeat sequences in the coding region of the gene (Sutherland, et al., 1995 and Zoghbi, et al., 2000) results in increased number of glutamine residues (36 -121) in expansion htt while in normal htt has 3144 amino acids with a molecular mass of around 348 kDa (Huntington’s Disease Collaborative Research Group, 1993). The HTT gene located on the short arm of chromosome 4 at 16.3 locus, initially labeled as IT15 (Interesting Transcript...
was found to contain an unstable CAG repeat in the open reading frame of exon 1. It has been described that normal subjects have a median of 19 CAG repeats (range, from 6 to 35) and was described instability of the trinucleotide repeats, whereas, nearly all patients with Huntington's disease have more than 40 CAG repeats (MacDonald et al. 1993). The gene typically contains 9-34 CAG repeats (Snell et al., 1993) and individuals carrying the HD mutations have more than 37 repeats (Gusella et al., 1996). An inverse correlation between the number of CAG repeats and the age of onset of HD and severity of expressed symptoms of HD has been reported (Trottier et al., 1994 and Brandt et al., 1996).

<table>
<thead>
<tr>
<th>Number of CAG repeats</th>
<th>Median Age of onset (Years)</th>
<th>Range in age of onset (85% C.I) (Years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>66</td>
<td>59-72</td>
</tr>
<tr>
<td>40</td>
<td>59</td>
<td>56-61</td>
</tr>
<tr>
<td>41</td>
<td>54</td>
<td>52-56</td>
</tr>
<tr>
<td>42</td>
<td>49</td>
<td>48-50</td>
</tr>
<tr>
<td>43</td>
<td>44</td>
<td>42-45</td>
</tr>
<tr>
<td>44</td>
<td>42</td>
<td>40-43</td>
</tr>
<tr>
<td>45</td>
<td>37</td>
<td>36-39</td>
</tr>
<tr>
<td>46</td>
<td>36</td>
<td>35-37</td>
</tr>
<tr>
<td>47</td>
<td>33</td>
<td>31-35</td>
</tr>
<tr>
<td>48</td>
<td>32</td>
<td>30-34</td>
</tr>
<tr>
<td>49</td>
<td>28</td>
<td>25-32</td>
</tr>
<tr>
<td>50</td>
<td>27</td>
<td>24-30</td>
</tr>
</tbody>
</table>

(Brinkman et al., 1997)

Another group believes that the disease onset is midlife in carriers of 40-50 CAG repeats, whereas individuals with the repeat length > 70 exhibit juvenile onset (Popovic and colleagues).

Normal htt is localized in the cytoplasm, whereas, the mutant form is found to be present both in the cytoplasm and nucleus (Trottier et al., 1995, DiFiglia et
al. 1995 and Sharp et al., 1995). The characteristic findings of progressive chorea and dementia are caused by severe neuronal loss, initially in the neo striatum and later in the cerebral cortex (Young, 1998). However, the brains of both humans with Huntington’s disease and transgenic mice with increased numbers of CAG repeats have intranuclear inclusions of huntingtin and ubiquitin of neurons of the striatum and cerebral cortex but not in the brain stem, thalamus or spinal cord, matching closely the sites of neuronal cell loss in the disease.

2.3 PREVALENCE OF HD

Albeit, the prevalence of Huntington found worldwide, the recent literature survey indicates some notable geographic differences and the exceedingly highest rates of prevalence of HD, reported in the isolated population in Venezuela (700 per 100000) (Warby et al., 2011), believed to have been introduced by the Spanish sailor between 1860 and 1870. Netherlands and the United Kingdom, historically, being high-prevalence regions. Studies have shown that emigration in the 18th and 19th centuries of individuals from Western Europe introduced the disease to North America, South Africa and Australia and elsewhere. In Eastern populations i.e. in China and Japan has been estimated to be 0.1 – 0.5 per 100000 (10^5) (Leung et al., 1992). Clinical diagnoses in the San-in area of Japan confirmed that the HD affected population is 0.65 per 100,000 (Nakashima et al., 1996). The prevalence rates for HD for the Middle East are likely to vary from 3 to 4 per 100,000 (Scrimgeour, 2009) and for Oman, with an Arab population of some 3,000,000 persons, it is likely that there are about 100 HD cases in Arabs country-wide, of which only about a dozen cases. The origins of HD chromosomes in the United States, Canada, South Africa, Australia, the Caribbean, the Indian Subcontinent and Venuzuela can be genealogically traced to European origins and having similar HD prevalence rates to Europe. The prevalence in African and American black populations is also considered to be lower than in white populations (Warby et al., 2011). In India, no juvenile HD is reported. However, 26 HD cases have been reported in the age group 11-20, out of which 4 reported cases were of Juvenile HD. There is hardly any data available for age group 0-10 in record (Quarrell et al., 2012).
2.4 MECHANISM OF NEURODEGENERATION

A number of cellular pathways have been shown to be affected by Huntington’s chorea due to the mutation in huntingtin protein which may result in misfolding of the protein. This impairs a number of cytoplasmic as well as nucleoplasmic events. Brain derived neurotrophic factor (BDNF) mRNA synthesis is reduced due to diseased protein, the former reduced (nearly 50%) (Zuccato and Cattaneo, 2007 and 2009) in peripheral cortex and transported along the cortico striatal tract to the medium spiny neurons (MSNs).

![Figure 2.3 Schematic representations of key cellular pathogenic mechanisms in HD (Zuccato Colleagues).](image)

The reduction BDNF level in cortical samples as well as in total brain from a large panel of model animals like R6/2, YAC, BAC-HD, N171-82Q knock-in mice and HD rats has been observed. This implies that the reduction of BDNF mRNA levels starts in early symptomatic stages and become more pronounced as the disease progresses (Zuccato et al., 2005 and 2010). Even, there are views that mutant huntingtin may affect BDNF transcription by altering the transcriptional activities of cyclic AMP response element binding protein (CREB), specificity protein (Sp1), CREB binding protein (CBP), TBP.
TATA Binding Protein associated factor 130 (TAF130) and other transcription factors that regulate the activity of BDNF promoters (Ciammola et al., 2007). Ultimately, it leads to reduction in BDNF vesicle transport which worsens the phenotype.

Binding of mutant huntingtin directly to mitochondria alters metabolic activity and motility within the neurons. The evidence came from ultrastructural studies in mitochondria isolated from HD cortical tissue which reveals abnormalities like depleted levels N-acetyl aspartate whose production is often regarded to reflect mitochondrial metabolism (Jenkins et al., 1993 and Molfitt et al., 2009). A number of biochemical studies on cells in an animal models, brain and peripheral tissue from HD patients have shown the decreased functions of several enzymes of oxidative phosphorylation (Tabrizi et al., 1999 and Thrushina et al., 2004).

Reduction in uptake capacity in the mitochondria from whole brain of mice, expressing full human mutant huntingtin and lymphoblasts of adult onset Huntington patients. Investigations of mitochondria isolated from lymphoblast of the juvenile HD patients reveals that these organelles are more sensitive to calcium uptake (Panov et al., 2002). A number of transcription factors that regulate the genes responsible for mitochondrial functions e.g. mutant htt binds p53 and results in the upregulation of Bcl2-associated X protein (BAX) and p53-upregulated modulator of apoptosis (PUMA) and refreshes transcription of PGC-1α (a transcriptional coactivator essential for mitochondrial biogenesis and respiration). Knock-out mice for PGC-1α show mitochondrial defects and striatal degeneration (Cui et al., 2006). Histone acetylases and histone deacetylases disrupted by the mutant htt protein leads to general transcriptional repression (Sadri-Vakili and Cha, 2006).

The pioneering work of Michael Hayden’s revealed the proteolytic cleavage of htt by caspases and hence blocking the proteolysis which may be beneficial to diseased HD mice. This gave rise to “toxic fragment hypothesis” that proteolytic cleavage of htt liberates toxic fragments which contain expanded polyQ results in their accumulation and activation of caspases in a cyclic manner. As result of this process, eventual demise of the cell occurs. Later,
it was reported by the same group that caspases is involved and important to
the proteolytic cleavage of the \textit{ht} studied by blocking caspasses in HD mice
(Graham et al., 2006). Evidence studied by Marian Difiglia and colleagues
showed that both normal an mutant \textit{ht} cleaved by \textit{calpain}, a family of calcium
dependent intra cellular cysteine proteases, which cleave the protein with in
\textit{NH}_2 terminal region. But the mutant is more susceptible to proteolysis and
generates and terminal fragments that are found in the cytoplasm as well as
nucleus of neuronal and non-neuronal cells (Kim et al., 2001).

\section*{2.5 MUTANT HUNTINGTIN PROTEIN AND INTRACELLULAR
ABNORMALITIES}

Though, the basic underlying defect in HD remains unclear, however, the
disease processes associated with HD are thought to be caused by a toxic
"gain of function," meaning that the mutated gene's protein (mutant
huntingtin) interferes with normal cellular functioning. For example, evidence
suggests that abnormal huntingtin may induce inappropriate, genetically
programmed nerve cell death (\textit{apoptosis}). In addition, many studies point out
that expansion of the amino acid glutamine within the huntingtin protein is
associated with accumulations / or inclusions of mutant huntingtin and an
associated protein (ubiquitin) within the nucleus of neurons in the basal
ganglia (i.e. striatum) and cerebral cortex. Evidence suggests that these
inclusions may form as cells attempt to inactivate the toxic huntingtin protein.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{basal_ganglia.png}
\caption{The Basal Ganglia of the human brain showing the impact of structure in
this region. The brain of a person with HD has bigger openings of lateral ventricles
due to the death of nerve cells in that region.}
\end{figure}
Caspase-1 is found to be active in the brain in HD patients and transgenic mouse model. Injecting of caspase inhibitor into the brains of transgenic mice associated with delayed disease progression, prolonged the life (Huntington's Disease collaborative research group, 1993). Delays in nerve cell damage and in symptom onset, with extended survival were also observed in genetically mutated mice models. Such findings suggest that caspase-1 may play a role in HD disease progression, thus by blocking the action of caspase-1 may reduce the implications for HD disease.

2.6 GENETIC TESTING FOR HUNTINGTON'S DISEASE

Huntington's disease is an autosomal dominant disease. Individuals have two copies of each gene, one gene inherited from the mother, and one gene inherited from the father. In autosomal dominant diseases, only one copy of the mutated or changed gene is sufficient to result in expression of the disease. Each child of an affected parent has a 50% chance of inheriting the diseased gene and may develop the symptoms of the disease disorder during the normal lifespan. If a child does not inherit the disease causing gene, the child will not develop symptoms of HD nor will that child transmit the gene that causes HD to future generations. HD affects males and females with equal frequency.

Literatures survey reveal that chorea movement was recognized as a neurological entity in the 17th century by the English physician Thomas Sydenham followed by a hereditary pattern of chorea was observed by Charles Waters in 1842 in families in New York. In 1872, though, the hereditary nature of chorea was noted by several doctors, George Sumner Huntington was the first to publish a lucid description of the symptoms of chorea and dementia in families in Long Island and, as a result, the disease came to bear his name. The discovery of the causative HD gene has stimulated research, and now the focus is on molecular mechanisms of disease.
2.7 TREATMENTS

Lot of work is going on to learn more about the inheritance patterns, pathophysiology, symptoms, and progression of HD and develop new or improved therapies. For example, investigators are evaluating whether excessive activation of glutamate may be reduced by blocking receptors of N-methyl-D-aspartate (NMDA), a similar neurotransmitter, possibly helping to halt abnormal nerve cell death. Studies are being conducted to assess the potential symptomatic and neuroprotective effects of riluzole (Rilutek®), an agent that is thought to moderate the effects of glutamate. Investigations are also evaluating other possible disease-modifying therapies, such as the use of antioxidants (e.g., coenzyme Q₁₀) or certain growth factors that may have a protective effect on striatal nerve cells (neurotrophic growth factors). In addition, experimental animal studies and clinical investigations are on-going to evaluate the safety and possible effectiveness of certain surgical techniques for HD. Such procedures include replacement of degenerated neuronal tissue through transplantation of human or pig fetal cells.

2.8 STRUCTURAL ASPECTS OF POLYGLUTAMINE

2.8.1 SPECTROSCOPIC STUDIES

a) Circular Dichroism

A study on synthetic peptide (Asp₂-Gln₁₅-Lys₁₂) by Circular Dichroism (CD - a low resolution technique) have proposed hydrogen bonded hairpin-like structure that aggregate to yield tightly packed β-sheets (Perutz, et al., 1994, 1995). Later, the same group proposed a β-helical nano-tube with 20 residues per turn (Perutz et al., 2002). In contrast, the CD (Nakano et al., 2009) measurement of synthesized water-soluble peptides - Ac-(Ala₄-Lys)₃-Gln₉-Lys-Ala₄-Lys-Ala₄-Lys-Ala-NH₂ - rich in alanine and lysine have 59% random coil and 41% α-helix contents. Likewise, CD measurements on the peptide, Tyr-Gly-Ala₂-Lys-Ala₄-Lys-Gln₁₇-Lys-Ala₄-Lys-Ala-NH₂, revealed that it is rich in random coil (67%), and α-helix content is only 26%. Altschuler et al., 1997 concluded that polyQ may adopt random coil conformation in aqueous solution with increasing chain length. In another such study, the peptides
containing a polyQ stretch, with or without interruptions, soluble in water at low concentrations exhibits β-structure and not random coil (Sharma et al., 1999). Surprisingly, the same authors have proposed that aggregation takes place via the formation of large intermolecular β-sheet structures and histidine interruptions of the polyQ stretch seems to favour intramolecular β-hairpin formation and reduces the potential to form large intermolecular β-sheet structure and aggregation. On the other hand Ross et al., 2003 have proposed a compact β-structure for polyglutamine.

CD and IR spectra (Tanaka et al., 2001) of sperm whale myoglobin, used as a carrier of polyQ stretches ranging from 12 to 50 repeats, showed that expanded polyQ region form anti-parallel β-sheet like structures. The properties of the β-sheets formed by expanded polyQ in the quasi-aggregate, a largely assembled non-fibrillar species of the protein, are quite different from those of regularly oriented and buried β-pleated sheets in fibril (Tanaka et al., 2003). The CD spectroscopic results on peptides K2-Qn-K2 for 5 ≤ n ≤ 44 in 10mM Tris-trifluoroacetate buffer (pH 7.0) show that the predominant conformation is random coil, but when n > 37, it has a tendency to form amyloid-like aggregates (Chen, et al., 2001 and 2002). In expanded conformations, stretch destablises the native protein conformation and promote aggregation through the formation of intermolecular β-sheets (Masino et al., 2002). The growing interest suggest that nucleation mechanism for aggregation, the frequency of nucleation and aggregate growth rate, observed both in cellular cultures and in vitro (in isolated form in test tube), increase with number of glutamines and protein concentration. However, other mechanism could also be responsible for aggregate formation. Thus, the conformational result on polyglutamine peptides is at variance.

b) Nuclear Magnetic Resonance Spectroscopic Investigations

On the basis of a NMR study of normal and expanded polyQ fused to glutathione S-transferase reveal the same random coil structures in both cases (Masino, et al., 2002). The structural aspects of soluble htt exon-1 fusion proteins (Thioredoxin) with 16 to 46 glutamine residues were found to
be altogether different (with no global conformation change when \( n > 36 \)) in the cytoplasm on the basis of NMR and CD spectroscopic results (Bennet et al., 2002).

The CD and NMR studies have been performed on glutamine peptides in different solvents. In short peptides i.e. tend to adopt a disordered structure and large peptides adopt a highly collapsed (Walters and Colleagues, 2009).

**2.8.2 X-RAY DIFFRACTION**

Perutz, et al. (1993), on the basis of X-ray diffraction study, has proposed that PolyQ chain could form antiparallel \( \beta \)-strands held together by hydrogen bonds between side chain and main chain amides and the antiparallel \( \beta \)-strands would either pair intramolecularly (form \( \beta \)-hairpin like structure) or intermolecularly (results in aggregate formation) when the length exceed a threshold value (i.e. > 35) - an unusual explanation in terms of side chain length. From thermodynamic considerations, the elongation of glutamine repeats beyond a certain length threshold may lead to a phase change from random coils to hydrogen-bonded hairpins thereby forming a ‘polar zipper’ (Perutz, 1996). X-ray diffraction of poly-L-glutamine (\( D_2-Q_{15}-K_2 \)) and the exon-1 peptide of \( htt \) with 51 glutamine repeats, have been reported to be similar by the same author. Later, they have proposed cylindrical \( \beta \)-sheets with \( \beta \)-strands normal to the fiber axis for poly-L-glutamine peptide - \( D_2-Q_{15}-K_2 \) (Perutz et al., 2002). Similar findings on kind of peptides have been reported by (Sharma et al., 2005). The accompanying study (Perutz et al., 2002) shows that the X-ray diffraction patterns of fibers of the exon-1 protein of \( htt \) together with those of poly-L-glutamine (\( D_2-Q_{15}-K_2 \)) exhibits reflection at 4.75 Å together with its second order and another at 8.3 Å together with its second and third orders and the interpretation of the one with the 8.3 Å is still unclear. They have observed that the glutamine repeat in the exon-1 protein of \( htt \) is followed by long repeats of prolines but the identity of its diffraction pattern with that of poly-L-glutamine shows that the prolines are not part of the ordered fiber structure. Recent X-ray diffraction study (Kim et al., 2009) proposed that the polyQ_{17} region in the \( htt \) protein adopts multiple conformations including \( \alpha \)-helix, random coil and extended loop and the
conformational flexibility of the region is a common characteristic of many amyloidogenic proteins. Like these CD spectroscopic results, X-ray diffraction studies on polyglutamine peptides are also at variance.

2.8.3 BIOLOGICAL MODEL

It has been hypothesized (Michelitsch and Weissman, 2000) that Gln and Asn-rich sequences are found in a variety of eukaryotic proteins to be crucial for several cellular functions. In fact, proteins rich in Q and N residues are involved in several processes as transcriptional activation, histone deacetylation and nuclear pore sieving. These residues also have the tendency to assemble into amyloid fibrils, leading to neurological disorders (Gatchel and Zoghbi, 2005 and Saunders, 2009) but how they assemble has not been explained yet. It is important to understand the structural basis for toxicity and the development of intrabody-based therapeutics for HD.

The aggregation model (Thakur and Wetzel, 2002) has been proposed and tested in the context of the htt exon-1 N-terminal fragment and the results seem to be consistent with an alternating β-strand/β-turn model. In cell model studies with interrupting proline residues in sequences revealed reduction in intra and inter molecular aggregate formation as well as toxicity (Poirier et al., 2005). A model study in vitro and in vivo with htt exon-1 either at N or C terminal expressing segment beyond the pathological threshold i.e. > 35 glutamine results in structural perturbation of neighbouring protein domains and increases the propensity of the chimeric protein for the formation of the aggregation hence towards toxic gain of function (Ignatova et al., 2006). Two types of aggregation process involving chimera composed of a globular protein (cellular retinoic acid-binding protein, CRABP) and HD exon-1 with tracts have been explored (Ignatova et al., 2007). PolyQ expansion reduces the nuclear export, where interaction between N-terminal htt fragments with nuclear pore protein translocated promoter region (Tpr) involved, causing the nuclear accumulation of htt protein (Cornett et al., 2005). The htt is primarily composed of HEAT repeats, and the biophysical data obtained on full-length htt support the conclusion that the structure of htt resembles that of other HEAT repeat proteins (Wei Li et al., 2006).
Although the structural basis that underlies the toxicity of proteins with expanded repeats is not clear, number of research groups have hypothesized that a variety of misfolded conformers, including monomers, oligomers, and fibrils, may be the toxic culprits.

2.8.4 POLYGLUTAMINE AGGREGATION, FIBRILISATION AND TOXICITY

The CAG repeats in the HD gene are expressed with 40 to 150 glutamine residues as an elongated huntingtin protein, whose function is unknown, is found in many cells in both neural and non-neural tissues (Young, 1998). *In vitro* studies *it has been shown by* electron microscopy that the N-terminal mutant htt forms aggregates which appear like a β-pleated sheet conformation but such aggregates are not observed when a normal stretch is present (Scherzinger et al., 1997). *This may imply the role of the other domains of the proteins in aggregation and fibril formation etc.* There is no direct relation between the amount of expanded huntingtin protein and the extent of neuronal injury (Martin, 1999).

Earlier study suggested that paternal imprinting is associated with both, a young age onset that occurs in maternal imprinting and a greater propensity to “major anticipation” (MA) i.e. a much younger age at onset than that in the affected parent (Ridley et al., 1988). Examination of the Huntington’s disease patients and families described the age of onset, that varies between families and between paternal and maternal transmission and rigidity is specifically associated with very early onset, major anticipation, paternal transmission, and young parental age of onset in particular juvenile onset can occur by normal variation in a family, which has a characteristically early age of onset (Ridley et al., 1991). The inheritance of a propensity toward juvenile onset via the affected male line could be due to an abnormal pattern of paternal genomic imprinting (Ridley et al., 1992).

A multi-domain misfolding mechanism in which the polyQ tract plays a critical role only in the late steps of aggregation towards the formation of the fibril has recently been demonstrated. This seems to be consistent with the idea that
crucial events of fibrillogenesis, in particular, the first steps are independent on polyQ thus correlating other 24 amyloids and polyQ proteins mechanism of fibrillogenesis, with the unique advantages of the latter to be a molecular hallmark for fibril formations (Thakur, et al, 2009, Ellisdon Collegues, 2006, de Chiara et al., 2009). This finding supports that the importance of adjacent tract of polyQ protein in modulating the aggregation pathway, and in consequence, the toxicity exerted by the protein. Nozaki et al., 2001 demonstrated the ability of polyQ flanking sequences to modulate aggregation by studying aggregation rates when the surrounding 17 amino acids of ataxin-2, ataxin-3, huntingtin and atrophin were added to the same polyQ tract. Duennwald and coworkers, 2006 employed a yeast model expressing huntingtinexon-1 to demonstrate that also polyQ toxicity is modulated by adjacent sequences, some exerting a protective effects, others increasing protein cytotoxicity (Duennwald et al., 2006). Only oligomeric species accounts for deleterious effects on the cell, protein context is likely to be responsible for modulating different types of intermediates aggregated species, with regard to isolate polyQ tracts, that undergo a simple aggregation mechanism from monomers to fibrils (Chen and Colleagues, 2002).

Many amyloid proteins carry out their physiological function in a folded structure, with predicted amyloidogenic regions well buried into the interior of the protein. A structural modification can expose such regions to solvent and switch protein from its physiological function to the pathogenic one. This can happen in vitro by promoting proteins unfolding conditions, as low pH, high pressure, high temperature (Shehi et al., 2003, Marchal et al., 2005, Booth et al., 1997, Valentine et al., 2005, Chiti et al., 1999, Stine et al., 2003). Bottomley and colleagues (Ellisdon Collegues, 2007) hypothesized that polyQ length modulates local stability and conformational dynamics, in agreement with previous cited observations.

The insoluble deposition of mutated htt in neurons observed in a transgenic model of HD suggested that toxicity is derived from its ability to form aggregates (Davies et al., 1997). Htt may shuttle between the nuclear and cytoplasmic compartments (Ross et al., 1999). Studies with Htt-Q_{95}, Htt-Q_{23}, and Htt-Q_{2} did not show any detectable aggregates while Htt-Q_{150} residues...
gave insoluble protein aggregates both in patient material as well as in vitro systems that become a hallmark of disease after the initial identification of nuclear inclusion bodies both in the mouse model and brain patient material (Faber et al., 1999). In HD and related expanded CAG repeat diseases, stretches of 36 or less are benign, whereas mutated, expanded forms with repeat lengths of 38 or more carry disease risk (Cummings and Zoghbi, 2000). After having tested 63 patients, it has been assessed that 25 had one expanded allele of 40 to 54 CAG repeats and the other allele in the normal range of 15 to 30 repeats (Law et al., 2001). The mechanisms underlying onset and progression of Huntington disease may differ as the disease receives two identical forms of a particular gene, one inherited from each parent, homozygote than in heterozygote (Squitieri et al., 2003). Inclusions are found in perinuclear and intranuclear locations because the β-tubulin binding property of huntingtin brings it to the perinuclear region, from which it readily gains access to the nucleus and the mutational glutamine expansion then promotes insolubility and results in an inclusion (Hoffner et al., 2001). It has been stressed that protein aggregates are initially restricted to the cytoplasm but over time they are observed within the nucleus and are associated with increased cell demise (Cowan et al., 2003).

Huntingtin protein length influences the ability of an expanded domain to alter gene expression suggesting that short N-terminal fragments of mutant htt might be responsible for the gene expression alterations observed in human HD brain (Chan et al., 2002). The slow forming aggregation of mutant huntingtin or the formation of nuclear inclusions could reduce the binding affinity of huntingtin for some interacting proteins (Yu et al., 2003) and the strategy to prevent the early neuropathological changes should focus on the interactions of transcription factors and proteins before the mutant protein form microscopic inclusions or aggregates. Supporting the existence of protein aggregates in cytoplasm and after some time in nucleus, htt aggregates sequester other expanded proteins in the cytoplasm and lead to disruption of axonal transport and accumulation of aggregates at synapses (Wyan-Ching Mimi Lee et al., 2004). One school of thought emphasized that both soluble and aggregated might harm neurons via non-exclusive
mechanism. Any evidence supporting the toxicity of one form should not be automatically regarded as evidence against toxicity of the other form (Michalik and Broehhoven, 2003, Sanchez et al., 2003). The disorders are due to toxic gains of function, as presumed, of mutant expanded proteins, a definitive mechanism for neurodegeneration, through the toxic gain of function of the mutant proteins in this group, currently remains obscure (Everett and Wood 2004). It has been suggested that peptides or small molecules that have the ability to block polyQ elongation and inhibitory properties may become viable therapeutic agents (Thakur et al., 2004). Human stem cell transplantation in a transgenic mouse model of HD has been recommended to test whether the cell replacement strategy is a viable therapy for the genetic HD mutation seen in the human population (Mc Bridge et al., 2004).

Nuclear intra-neuronal inclusions (NII) are often observed and reported in or near affected brain regions in HD patients (DiFiglia et al., 1997) and in animal and cell models (Reddy et al., 1999). Formation of neuronal inclusions in non-CNS tissues has been analysed in order to prevent the aggregation in vivo (Sathasivam et al., 1999). Aggregates of peptides prepared in vitro and introduced into cells in culture are benign when delivered to the cytoplasm, but highly toxic when delivered to the nucleus (Popiel, et al., 2004). Whereas, the other possibility proposed that htt aggregation in the nucleus could represent benign sequestration of a protein fragment resistant to proteolysis (Ordway et al., 1997). What mechanisms are taking place in the nucleus when aggregate introduced into the nucleus to produce cataclysmic results is yet unknown? Thus, the length of the peptides which is toxic at the cytoplasmic or at nuclear level may be different. It is evident that mutant htt is localized in the nucleus and cytoplasm may thus affect a variety of cellular functions which causes neuronal cell death. Although, the nuclear localization of mutant htt plays a critical role in gene transcriptional dysregulation, how mutant htt accumulates in neuronal nuclei remains a mystery. Transgenic mouse models of HD clearly show that N-terminal htt fragments can accumulate in neuronal nuclei in the brain and cause severe neurological symptoms (Havel et al., 2011). The kinetics aggregation (Scherzinger et al., 1999 and Chen et al., 2001) of containing peptides

25
depends on repeat chain length, which qualitatively mirrors the repeat length
dependence of disease risk, even though the length of tract correlates directly
with the age of onset and severity of symptoms in the diseases.

The sequences have a random coil conformation when solubilised by highly
soluble carriers, however, expanded regions in htt have increased tendency
for aggregation and destabilize the native protein structure (Masino, 2004).
The common characteristic of disease is the formation of intranuclear protein
aggregates that contain the expanded glutamine repeats and show a granular
or fibrillar morphology (Wanker, 2000). The hallmark of HD and other
diseases is the formation of insoluble protein aggregates in affected neurons.
The lengths in the pathological range i.e. ≥ 40 are insoluble, whereas,
fragments carrying non-pathogenic repeat lengths are soluble. In vitro N-
terminal mutated htt form aggregates which have been interpreted as a fibrillar structure without emphasizing on ϕ, ψ values (Orr, 2001).

Varying morphologies of fibers and amorphous aggregates generated in vitro
for containing peptides and proteins suggesting that the pathway of fibrillization may be complex.

There are evidences that when htt contains a mutated, expanded stretch, it
translocates from its site of synthesis in the cytoplasm into the nucleus, where
mutant htt accumulates and induces neurodegeneration. Here, the important
observation is that the steps that are necessary to translocate mutated htt
from the cytoplasm into the nucleus are not known. Another hypothesis has
been made that full-length mutant htt is sufficiently large that it would have to
be actively transported across the nuclear envelope to enter the nucleus.
Nuclear import may be mediated by a nuclear localization sequence (NLS)
with htt (Bessert et al., 1995). The nuclear import occurs more readily, if
mutant full-length htt is first proteolysed in to smaller fragments (Goldberg et
al., 1996). The studies with yeast has shown that aggregated fragments of
polyQ expanded htt protein, the causative factor in Huntington’s disease, are
not particularly toxic to cells expressing them (Krobitsch and Lindquist, 2000).
Mutant htt acts within the nucleus to induce neurodegeneration but
intranuclear inclusions may reflect a cellular mechanism to protect against htt-
induced cell death (Saudou et al. 1998). In order to determine whether expanded \textit{htt} induces neuronal toxicity, the expression of the full length of \textit{htt} with 16, 48 or 89 polyQ repeats in a rat hippocampal neuronal cell (HN333) has been examined (Liu, 1998) and concluded that mutated \textit{htt} with 48 or 89 repeats stimulated c-Jun amino-terminal kinases (JNKs) activity and induced apoptotic cell death in HN33 cells, while expression of normal \textit{htt} with 16 repeats had no toxic effect suggesting that activation of JNK, induced by mutated \textit{htt} takes place several hours prior to apoptotic cell death. In the cell model, Q\textsubscript{35}-GFP and Q\textsubscript{80} GFP, the neuron would survive as long as the repeat length is small enough that the rate of aggregation is slower than the rate of degradation (Moulder et al., 1999). PolyQ aggregation, proteasome activity, and cell death in neural SH-SY5Y cells that were stably transfected with green fluorescent protein (GFP) containing 19, 56, or 80 glutamine repeats have been analysed and reported that the ability to elevate proteasome activity is impaired in 56 and 80 glutamine GFP-transfected cells and precedes formation of stable GFP aggregates (Ding et al., 2002). PolyQ enhancer (pqe-1) proteins containing the Q/P-rich domain and the charged region are critical for protecting neurons against neurotoxicity effects of expanded (Faber et al., 2002). The CA150 (the Gln-Ala repeat transcriptional activator) could be involved in HD pathogenesis and its accumulation within cortical and striatal neurons of HD brain tissue also suggesting that it could interfere with the transcription of genes essential to neuronal survival (Holbert et al., 2001). The theory about HD is not shortened instead lengthened. Another report suggests that a formic acid-resistant oligomer, polymeric aggregate of expanded, is present in cortical nuclei of cerebral cortex, but not in cerebellum (Luchi et al., 2003). In addition to the monomeric \textit{htt} fragment, the presence of an expanded containing protein of size correspond to dimer has been suggested in the dissolved aggregates (Hazeki et al., 2000). Full-length huntingtin protein confers a selective accumulation of mutant huntingtin aggregates in the axons of striatal projection neurons, which are preferentially affected in HD, is specifically associated with axonal degeneration and suggests that HD pathology originates in axons (Li et al., 2001). Biologically active multiple small molecules that target to reduce/inhibit \textit{htt} aggregation and toxicity have been
tested (Desai et al., 2006) for important therapeutic target. An isolated ubiquitin protein has been suggested (Miller et al., 2007) as potential inhibitors of aggregation in vivo (in the living cell). Expansion does not necessarily lead to aggregation formation, and that the enhanced kinetics may affect the nuclear function ataxin-1 (Hilde et al., 2008). Different views are still expressed on the role of basal ganglia and striatum; one of the studies indicates that the striatum encompasses one or several circuits, which are involved in linguistic and non-linguistic rule application (Teichmann et al., 2005). The pathological abnormalities are restricted to the brain, with preferential vulnerability in the striatum and cerebral cortex, the intense aggregation of -EGFP (enhanced green fluorescent protein) has also been observed in hypothalamus neurons (Kotliarova et al., 2005). Cortical dysfunction has been considered an early symptom of HD in the regions other than the striatum (Andre et al., 2006). Some of the research studies concluded that an assumption had largely been accepted that this indeed responsible as a true pathogenic agent in neurological disorders, but until now remain unproved (McLeod et al., 2005). The mutant protein in Huntington’s disease due to repeat leading to a polyQ strand at the N-terminus confers a toxic gain of function (Walker, 2007). Proteolytic cleavage of the polyQ region from the intact protein, known to be important for aggregate formation in Huntington’s disease (Wellington, 2000), may play more general role in the initiation of other, if not all, disorders. If this were the case, it would clearly influence the choice of further model systems. This may imply that the length dependence of disease is not related to a conformational change in the monomeric states of expanded sequences. The simple nucleated growth models for htt aggregation suggest that, because nucleation is a very rare event, and elongation is much more efficient than nucleation, each cell should never contain more than one aggregate (Wetzel, 2006). It has been observed that in the neurodegenerative disease where the CAG repeat code for protein gains some disease-producing function when glutamine copy number increases as little as one above the normal level can result in disease. Green (1993) has hypothesized that the reiterated glutamines one involved in protein aggregation by cross linking a glutamine residues to polypeptides containing lysyl groups and that this is slow process
critically depend upon the number of glutamine residues. Perutz et al. (1994) have similarly suggested that protein aggregation could be the disease-causing process but hypothesized that the mechanism of aggregation is that the glutamine repeats act as polar zippers. Either process is likely to act in a dominant manner and may be quite independent of the normal function of the repeat containing protein. If protein aggregates accumulate with time than this could provide a molecular basis for the copy number/age-of-onset relationship. PolyQ tracts would form two antiparallel β-strands stabilized by hydrogen bonds leading to multimerisation and aggregate formation. In support of this hypothesis, Stott et al. (1995) have demonstrated that the addition of a polyQ tract of 10 residues in to chymotrypsin inhibitor 2 resulted in its multimerization. However, subsequent crystal structure analysis failed to provide support for multimerization in the presence of hydrogen bonds between β-strands. Chen et al. (1999) have solved the crystal structure of the dimer of a mutant with four glutamines using the molecular replacement method taking the wild-type monomer as a search model. They have found that the structure of each half of the dimer is found as same as that of the wild-type monomer, except around the glutamine insertion. The authors have proposed that the components of the oligomers are held together by hydrogen bonds between the main-chain and side-chain amides of the glutamine repeats. An in vitro aggregation assay was developed by Scherzinger et al. (1997) and used to examine the aggregation properties of polyQ peptides. They have found that the N-terminal fragment of htt with 51 glutamines formed amyloid-like fibrils. Yet, the additions of 243 amino acids form the glutathione s-transferase protein blocked the formation of these aggregates. Furthermore, full-length htt with an expanded polyQ tract did not aggregate spontaneously in vitro. Thus, these data indicate that if a polyQ tract can drive multimerization, first it must be cleaved from the intact protein. Peptides generated by proteolysis of the parent proteins are water insoluble and aggregate in vitro (Temussi, et al., 2003) and the fragments containing expanded polyQ tracts form insoluble amyloid-like aggregates both in vitro and in vivo (Scherzinger, et al., 1997). PolyQ aggregation can occur independently in the presence of other cellular components, which are a function of protein concentration, time and glutamine repeat-length, with a
good correlation with the pathogenic threshold (Scherzinger, et al., 1999). The polyQ disorders are associated with the formation of neuronal nuclear inclusion (NI) (Mangiarini, et al., 1996), which are initially form at the axons and dendrites of nerve cells in specific areas of the human brain and are making significant changes in cell structure as well as with other proteins, ultimately become toxic to the nerve cell.

Other emerging data also suggest that microscopic nuclear aggregates may not be pathogenic. It has been very difficult to show neuronal death in an animal model of HD in which there are nuclear aggregates (Davies et al., 1998). Transfected cells with cytoplasmic but not nuclear aggregates experience heightened cell death (Hackam et al., 1998). A Transgenic model using a polyQ expansion to cause an unrelated cytoplasmic protein to translocate to the nucleus and aggregate did not show any neuronal death (Ordway et al., 1997). Cell culture study has suggested that nuclear but not aggregated htt is toxic (Saudou et al., 1998) and found that inhibiting ubiquitination and consequent formation of aggregates in the cell nucleus increased rather than decreased the toxic effect of polyQ expanded htt expressed in transfected striatal neurons.

Many studies have supported the hypothesis that the aggregates of peptides containing a nuclear localization signal are localized to nuclei and lead to dramatic cell death whereas amyloid fibrils of a non-peptide are non-toxic, whether localized to the cytoplasm or nucleus. In one of the study, it has been shown that the aggregation of a protein containing a polyQ stretch of pathological length is abolished when its expression is targeted to the endoplasmic reticulum, whereas, when it is transported outside the endoplasmic reticulum, it regains its ability to aggregate (Rousseau et al., 2004). The initial process of polyQ aggregation in vivo has been examined and ended with the result that tracts were of a soluble form during a lag period and then formed insoluble complexes when the tract is tremendously increasing (Kimura et al., 2001).

The role of aggregates in the disease mechanism have been carried out more directly by preparing protein aggregates in vitro from simple peptides
and introducing them into the cytoplasm or nucleoplasm of cultured cells (Yang et al., 2002). These authors have suggested that the nuclear localization of an aggregate of a short, Q_20, peptide is just as toxic as that of a long peptide, supporting the notion that the influence of polyQ repeat length on disease risk and age of onset is at the level of aggregation efficiency. They have stressed that aggregates remaining in the cytoplasm are benign, but the mechanism is not known, cytoplasmic aggregates occasionally migrate into the nucleus, with cataclysmic results. Synthetic monomer peptide functions as the critical nucleus for aggregation at different concentration and the fibril formation proceeds via linear additions of single molecules, after nucleation events consisting of a random coil to β-sheet transition within individual monomer (Chen and Berthelier, et al., 2002). They have demonstrated the results from Circular Dichroism (CD) spectra which indicate that aggregates are dominated by β-sheet which is consistent with an amyloid-like substructure. The peptides, K_2-Q_{90}-(PG-Q_{30})_3-K_2 and K_2-Q_{197}-(PG-Q_{30})_3-K_2 undergo spontaneous aggregation as efficiently as a Q_{40} sequence i.e. K_2-Q_{40}-K_2, whereas the corresponding peptides K_2-Q_{70}-(PG-Q_{30})_3-PG-Q_{30}-K_2 and PGQ_8 i.e. K_2-Q_{70}-(PG-Q_{30})_3-K_2-PG-Q_{30}-K_2 aggregates much less (Thakur and Wetzel, 2002) and the peptide P_0-GQ_8 (D-proline) sequence aggregates more efficiently than the peptide with L-prolines, consistent with β-turn formation in aggregate structure. The data suggest that the polyQ peptides adopt a structural model comprised of alternating beta-strand/beta-turn elements, with an optimum of seven or eight glutamine residues per β-strand.

Based on the similarities between htt fibrilization and that of other amyloid-forming proteins, HD toxicity is governed. The process of htt aggregation is complex and involves a multi-step process, involving monomers, soluble and insoluble oligomers and fibrils, and ultimately leading to inclusion body formation, therefore, toxic oligomeric species may accumulate around mitochondrial and endoplasmic reticulum and influence the ability of htt to dysregulate [Ca^{2+}]homeostasis (Rockabrand et al., 2007). The expression of mutant htt selectively impairs the mitochondrial Complex III activity, which in turn, the deficits in the mitochondrial Complex III promote the accumulation of htt aggregates through the inhibition of proteasome activity (Fukui and Moraes, 2007).
The rate of aggregate formation in vitro directly correlates with repeat length: the longer the polyQ tract, the faster the aggregation rate (Scherzinger, et al., 1999). The aggregation of the protein htt is dependent on the length of a polyQ region within the htt protein sequence and patients with longer htt polyQ regions (> 35 glutamines) demonstrate increased htt amyloid fiber formation as well as an increasing risk of neuron death, cognitive dysfunction, and atrophy of motor functions (Zoghbi and Orr, 2000). Mutant htt with > 40 CAG repeats gains a toxic function and induces death in subpopulations of neurons in the striatum and cortex (Zoghbi and Orr, 2000). A polyQ aggregate is a generic attribute of polypeptide chains, especially of main-chain atoms that are common to all protein sequences (Dobson, 2004). Aggregation of protein is believed to be one of the most characteristic features of aggregation diseases which give rise to the deposition of proteins in the form of amyloid fibrils and plaques and the deposition can form in the brain in case of neurodegenerative diseases, the quantity of such aggregates can be almost undetectable in some cases. Analysis has been carried out on interrupting the stretch by inserting Proline, which can cause great alterations in protein conformation, whether disrupting the toxic conformation of the protein can alter its aggregation propensity and cytotoxicity (Popiel, et al., 2004). They have showed that insertion in such a manner indeed disrupts the secondary structure, leading to suppression of protein aggregation both in vitro and in cell culture, and reduction of cytotoxicity in correlation with the number of proline interruptions. Further, it has been shown that short polyQ stretch with a proline interruption is able to inhibit aggregation of the expanded polyQ protein in trans. These results show that a gain in toxic conformation of the expanded is essential for its aggregation and cytotoxicity. Short amino acid sequences, FLAG-epitope (DYKDDDK), flanking the polyQ region of htt exon-1 can determine the benign or toxic character of a polyQ expansion protein and the flanking sequence can direct misfolding to at least two morphologically distinct types of polyQ aggregates (Duennwald et al., 2006) of very tight and amorphous nature. It is stressed that the toxicity is strongly associated with aggregation of expanded but not associated with the short polQ, which did not form aggregation (Merin et al., 2002). Flanking sequences, when present on the same polypeptide chain as the tract, can
convert a toxic protein to a nontoxic state and a nontoxic protein to a toxic state. In their subsequent study, they have demonstrated that the same flanking sequences can also exert their protective or toxic function in trans (when present on different polypeptide chain) underlining their significance to toxicity. Further it has been shown that like the flanking sequences, the Gln-rich interacting proteins have the capacity to transform a nontoxic expanded htt exon-1 protein to a toxic one and toxic one to a nontoxic one. As various levels of study suggests that the truncation of htt is needful to aggregate and to localize to the nucleus, the cellular localization of full-length and truncated htt fragments in transient transfection experiments in cell culture have been conducted and found that N-terminal truncations of htt with expanded repeats form nuclear and cytoplasmic aggregates, which can increase the tendency to cellular toxicity (Cooper et al., 1998). Evidence suggests that aggregates of truncated huntingtin are toxic and likely to translocate to the nucleus (Mukai et al., 2005).

Analysis of specific neurons in Caenorhabditis elegans, developed for neuron-specific pathogenesis, revealed that only at the threshold length, but not at shorter lengths, proteins can exist in a soluble state in certain lateral neurons or in an aggregated state in motor neurons of the same animal (Brignull et al., 2006). Generation of containing N-terminal htt fragments by proteolysis leads to the accumulation of toxic peptides (Ellerby et al., 2006) that also form aggregates in the nucleus in neuronal processes, which include axons and dendrites (DiFiglia et al., 1997). An intracellular htt antibody, EM48, which can preferentially react with mutant htt (Gutekunst et al., 1999), has been developed (Wang et al., 2008). This intrabody reduces the cytotoxicity of N-terminal mutant htt, the formation neuropil aggregates and the neurological symptoms of HD, when it is expressed in neurons. The findings have suggested that the intrabody specifically targets mutant htt with abnormal conformation and can serve to suppress the cytoplasmic neuropathology of HD and thereby differentiating the cytoplasmic from the nuclear effects of mutant htt. A variety of complementary approaches have been used to demonstrate that CHIP - the C-terminal heat shock protein (Hsp70) – interacting protein, suppresses aggregation and toxicity in
transfected cell lines primary neurons, and a novel zebrafish model of disease (Miller et al., 2005). Through this study, CHIP has been considered to be a critical mediator of the neuronal response to misfolded/ aggregated protein and represents a potential therapeutic target for neurodegenerative diseases.

A bacterial artificial chromosome-mediated transgenic mouse model (BACHD mice) expressing full-length human mutant huntingtin (mHTT) was established (Gray et al., 2008) and it exhibits progressive motor deficits and late-onset selective neurodegeneration in the striatum and cortex, consistent with the findings in the YAC128 animals. The BACHD mice are also inherently well suited for therapeutic trials. A proposed mouse model (Mangiarini, 1996 and Reddy, 1998) discussed the expression of polyQ containing proteins in the central nervous system is sufficient to cause neurotoxicity.

Though the number of lines of evidence point to the critical involvement of aggregation in the disease process, what is influenced in repeat length that dramatically changes its structure as aggregation and neurotoxicity character has remained obscure.

To understand the molecular basis of the processes of aggregation, fiber formation and toxicity, a detailed knowledge of the structural properties is essential. In addition, the characterization of the three-dimensional structure of proteins would yield important information on their function and on interactions with cellular partners, thus providing useful insights into the mechanism of neurodegeneration.

However, it is still challenging to obtain fully atomistic information about the structure of amyloid fibrils oligomers. Nonetheless, X-ray structures of 3D crystal of small peptides (Nelson et al., 2005 and Sawaya et al., 2007), combined with information from techniques such as solid-state NMR (Bu et al., 2007, Jaroniec and Colleagues, 2004, Gordon and Colleagues, 2004, van der Wel and Colleagues, 2007) cryo-electron microscopy (Sache et al. 2006, Jimenez et al., 2002), transmission electron microscopy (Vitrenko et al., 2007, Walters and Murphy, 2009), electron paramagnetic resonance (Cobb et al., 2007).
Mutational approaches (Koo and Colleagues, 2008), size-exclusion chromatography (Walters and Murphy, 2009), atomic force microscopy (AFM) (Elam et al., 2003) and hydrogen exchange (Kardos et al., 2005) experiments provide insights on molecular conformation of different oligomeric and fibrillar forms (Nelson and Eisenberg, 2006). To understand the details of the molecular mechanisms and intermediate structure involved in the aggregation process may counteract as essential to prove misfolding of diseases. An approach which can help for elucidating the structural information of polyQ proteins with experimental techniques such as computational methods, particularly, simulation studies have demonstrated to be a powerful tool to complement experimental studies aimed at understanding protein aggregation in several amyloid proteins (Caflisch, 2006, Cellmer et al., 2007, Ma and Nussinov, 2006, Moroni and Colleagues, 2009).

The emphasis has been on the conformation and aggregation behaviour of short polyQ homopolypeptides. The conformation and aggregation behaviour of these peptides will be different from the aggregation behaviour of peptide essential for the onset of Huntington’s disease. Further, the conformational behaviour of the polyglutamine peptide with minimum threshold or more will be different when it is a part of the htt protein. As polyglutamine contains amide linkage in the side chain, therefore, the conformation of the glutamine peptides may involve (i) interactions between the peptide bond and side chain amide linkage (ii) interactions between the peptide bonds (iii) interactions between the side chain amides. Therefore, this study deals with the systematic conformational behaviour of polyglutamine peptides of varying chain length and their aggregation behaviour.

### 2.8.5 SIMULATION STUDIES ON POLYGLUTAMINE

A number of computational studies have proposed different structural models (Munoz and Serrano, 1997). Molecular modelling studies hypothesized that could be a β-hairpin (Perutz, 1996). Starikov et al., 1999 reported: (i) preference of β-hairpin state over an extended state by using Flory-Huggins mean-field lattice model, (ii) whereas, all-atom energy minimization of
expanded glutamine peptides with implicit solvation produce a β-hairpin structure (iii) transition from random coil to β-hairpin structure by using AMBER software. Simulation studies of Chymotrypsin Inhibitor 2 (CI) chimeras containing glutamine inserts of chain length 4, 6, 8, 10, 20, 30, 35, 40, 50, 60 and 80 between residues 43 and 44 suggests that for shorter glutamine unfolded state is rescued by the formation of domain-swapped aggregates. With increasing insertion length, the pathogenic threshold (35-40) also increases (Barton et al., 2007). Substitution of methionine 40 by MG₃SG₄SG₅M, MGQ₂GM, and MGQ₁₀GM in wild-type protein Chymotrypsin inhibitor 2 as well as CI₂ insert mutants results in that polyglutamine adopt a structure corresponding to an ensemble of extended random coil conformations (Finke et al., 2004). In MD simulations of glutamine peptides of chain length 20, 40, and 80 with alternate chirality, a more disordered random-coil structure has been preserved as compared to L-glutamine peptides (Armen, et al., 2005).

An ensemble of four possible motifs, parallel and anti-parallel β-sheets, α-helix, and π-helix conformations have also been proposed (Lathrop, et al., 1998). It may be pointed out here that the π-helix is very rare in proteins (Linus Pauling et al., 1951). Monoi et al., 1995 and 2000, by molecular mechanics study, have claimed the formation of μ-helix (not observed yet) by large glutamine peptides which are cation selective. The pore size of the helices increases from 3₁₀ to α-helix to π-helix and in μ-helix 37 residues are claimed per turn thus the pore size will be too much. The structure proposed by Monoi et al., is self contradictory by their own results. If μ type of helix is found, it will not be cation selective and it will lead to lysis of the cells. Possibly, it is due to this polyQ channels may be the toxic element in mutant huntingtin protein (Kim and Tanzi, 1998).

The MD simulations of the mutant peptide-PGQ₃ forms a four-stranded antiparallel β-sheet and structures at low temperature (Khare et al., 2005) and all residues adopt β-strand backbone dihedral angles and the polypeptide chain coils around the central helical axis with 18.5±2 residues per turn. They have also found that mutant peptides with proline-glycine insets show formation of antiparallel β-hairpins in their ground state.
Stork, et al., 2005 and Numata, 2005 have claimed that the three-coiled \(\beta\)-helices are unstable, proposed by Perutz et al. 2002, but stable at a triangular shape with 18 residues per coil. The individually unstable two-coiled triangular \(\beta\)-helices become stabilized upon dimerization, suggesting that seeded aggregation of Huntington amyloids requires dimers of at least 36 glutamine repeats (or monomers of ~ 54 glutamine) for the aggregation of sufficiently stable aggregation nuclei. Inhibition of polyQ aggregations by substituting prolines at various positons in polyQ have also been studied by MD simulations (Nakano et al., 2009).

On the basis of the discontinuous molecular dynamics simulations (DMD) of glutamine peptides of chain length 12, 24, 48 and 96, Nguyen et al. 2004 have observed that the fibril formation is nucleation dependent, function of temperature and peptide concentration and proceeded by the formation of amorphous aggregates, which are not fibrils or nonfibrillar \(\beta\)-sheets. Conformational ensembles for two short polyglutamine peptides is disorderd (Wang et al., 2008) and the compactness and magnitude of conformational fluctuations increase with chain length. Similar type of simulation have been conducted on systems containing 16, 24, 32 and 48 polyQ chains and found that when the side chain hydrogen-bonding interactions were turned on, the peptides folded into amorphous aggregates at low temperatures, ordered aggregates with significant \(\beta\)-sheet character at intermediate temperature, and random coils at high temperature structures (Marchut et al. 2006 and 2007). Merlino et al., 2006 observed that in polyglutamine peptides shorter than two full \(\beta\)-helix turns are unstable and collapse toward irregular structures, whereas, longer \(\beta\)-helix models, containing more than 40 residues, achieve a dynamic regular structure. Surprisingly, in one of the study polyQ peptide adopts \(\alpha\)-helix structure (Bhattacharyya et al., 2006 and Slepko et al., 2006). The polyQ stretches in some diseases-causing proteins have \(\alpha\)-helix structure in their native form, with increasing propensity for a \(\beta\)-sheet conformational transition upon expansion above 40 repeats (Richardson et al., 1989 and Zagorski et al., 1992).
2.8.6 THERAPEUTIC APPROACHES TO INHIBIT AGGREGATION

The hypothesis of the abnormal aggregates of the NH$_2$-terminal region of the HD protein accumulate selectively in neurons that degenerate in HD has been tested analysing immunohistochemistry in postmortem brain tissue controls, HD patients with juvenile and adult onset HD (DiFiglia et al. 1997). Western blot analysis carried out on nuclear extracts from the cortex of controls and juvenile HD patients suggest that an NH$_2$-terminal fragment of mutant $htt$ translocates to the nucleus and contributes to the formation of Neuronal Intranuclear Inclusions (NILs).

The molecular aspects of polyQ aggregate formation are still poorly understood, but a commonly held view is that limited proteolysis may generate aggregation-prone fragments of the mutant protein in at least some glutamine-repeat diseases (Merry et al. 1998). Aggregation may occur preferentially in the nucleus. Proteasomes may contribute to the aggregation process and toxicity by releasing polyQ -rich fragments having a greater tendency to aggregate than full-length polyQ proteins (Venkataraman et al., 2004). Although, several lines of evidence have suggested that polyQ -polyQ interactions are important molecular mechanism for their aggregation some literatures claiming that the aggregation formation is initiated by the full-length protein (Perez et al. 1998) and not by a polyQ -containing fragment of the disease protein. The authors explained that once aggregation is initiated because of amide functionality in the side chain. Short polyQ containing proteins may not usually change their conformation or form aggregates and their aggregate formation could be highly dependent on molecular chaperone(s), whereas, the presence of a long polyQ tract in a disease protein may cause chaperon-independent aggregate formation and may induce the aggregate formation of other polyQ containing proteins (Kimura et al., 2002). Subsequently, it has been stressed that the polyQ aggregates formed independently of Hsp104, are required for Hsp104 to efficiently produce more aggregates (Kimura et al., 2004). After having engineered $Drosophila$ to express different peptides, it has been found that the expanded chains alone are intrinsically cytotoxic and cause neuronal
degeneration and early adult death. This intrinsic toxicity depends on cell type and length of the glutamine peptides. The inclusion of other amino acids modifies the polyglutamine structure and aggregation and thus reduces toxicity (Marsh et al., 2000 and Slepko et al., 2006).

At present, the emphasis is on designing of inhibitor that to reduce or stop target protein – protein interactions for aggregate (Kazantsev et al., 2001). The role of aggregation in pathogenesis is still a debatable subject. Even, there are views that aggregation and cell death are separate phenomena (Nagai et al., 2000).

Inhibition of digestion of huntingtin by caspase 3 and 6 reduced apoptotic rate in cell models suggest that caspase inhibitors may provide a potential new therapeutic approach. Identification of compounds that selectively alter intracellular interactions and metabolism of pathological-length, polyQ domain proteins could be an effective therapeutic strategy.

There are reports that Hsp70 and Hsp40 chaperones can shield toxic forms of proteins and direct them into non-toxic aggregates that prevent or delay disease initiation or slow disease progression (Muchowski et al., 2000). The Hsp70 or glutamine-rich proteins, such as CREB-binding proteins (CBP), essential transcriptional co activators, are sequestered irreversibly by aggregates and that the resulting cellular toxicity is caused by the loss of these proteins for normal cellular function (Stenoien et al., 1999). This has not been established, rather it demonstrates that the surface of the growing aggregate binds and releases Hsp70 in its role as a molecular chaperone to prevent the accumulation of unfolded proteins (Kim et al., 2002).

Suppression of cellular toxicity of htt by HSP40 and HSP70 can also be due to inhibition of caspase activation, rather than an effect on aggregation (Zhou et al., 2001). Inhibition of huntingtin aggregation or stimulation of the natural clearance of accumulated disease protein by small molecules is an effective therapeutic strategy (Waelter et al., 2001). Progressive deafferentation/efferentation and its consequent decrease in brain derived neurotrophic factor (BDNF) in conjunction with N-methyl-D-aspartic acid (NMDA) receptor supersensitivity, may initiate a cascade of downstream
effects that lead to cell death in HD (Cepeda et al., 2003). A drug-like small molecule, C2-8, has been identified in a yeast-based aggregation assay and observed to partially inhibit polyQ mediated aggregation in cell and brain slices culture (Zhang et al., 2005). HSP40 and HSP70 have been expressed with protein (Chai et al., 1999 and Jana et al., 2000) and it has been demonstrated that among several chaperones, some are co-localized with the aggregates and transient overexpression of these chaperones reduces the aggregate formation as well as cellular toxicity caused by expanded polyQ tracts. Proteasome inhibitors and heat shock increases the proportion of HD exon 1-expressing cells with inclusions that may be enhanced in poly Q diseases (Wyttenbach et al., 2000). In contrast to HDJ-1 and HSP70, HSP27 did not redistribute to inclusion. Intracellular distribution of endogeneous and exogeneous HSP27 is unable to suppress polyQ aggregation and neither to bind to htt exon-1 with a polyQ expansion nor redistribute to inclusions. This emphasize that HSP27 protects against polyQ toxicity by acting on cell survival/death pathways independent of aggregation suppressions (Wyttenbach et al., 2002).

The small molecule C2-8 has been evaluated as a therapeutic lead compound in the R6/2 mouse model of HD and was found to haveno toxic effect, reduces huntingtin aggregate size in vivo and modest neuroprotective (Chopra et al., 2007). The (-) -epigallocatechin-3-gallate (ECGC) and related polyphenol have been identified as potent inhibitors of mutant htt exon-1 protein aggregation in vivo. ECGC suppresses the toxicity and aggregate formation in yeast and fly model (Ehrnhoefer et al., 2006).

It is also possible to prevent mutant Htt aggregation in vitro using specific monoclonal antibodies corresponding to the polyQ repeat (Heiser et al., 2000) and a number of chemical compounds like Congo red, thioflavin S and Chrysamine G. Benzothiazole derivatives have also been proposed as potent inhibitor of HD exon-1 aggregation in vitro and in cell culture model systems of HD as they bind to polyQ (Heiser et al., 2002).

The polyQ Binding Peptide 1 (QBP1) prevents polyQ oligomerisation and aggregation either by altering the toxic conformation of the expanded polyQ
stretch, or by simply competing with the expanded polyQ stretches for binding
to other expanded polyQ proteins (Nagai et al., 2003) but the molecular
mechanism by which the binding of QBP1 to the expanded polyQ stretch
prevents aggregate formation. QBP1 prevents the initial β-sheet
conformational transition of the expanded polyQ protein monomer triggers
cytotoxicity (Nagai et al., 2007) and proposed that the toxic β-sheet
conformational transition of disease-causing protein monomers, and not
simply the aggregation process itself, could be a target for potential therapies
for polyQ diseases.

*In vitro* cell mode of HD, over expressed Dnaj-like protein (MRJ), a class of
molecular chaperon which is highly enriched in the central nervous system,
effectively suppressed polyQ dependent protein aggregation, caspase
activity, and cellular toxicity (Chuang et al., 2002). The disruption of
aggregation in the inducible rat phaeochromocytoma (PC12) cells strongly
correlates with suppression of neuronal degeneration *in vivo* and thus
providing a rapid and effective therapeutics of polyQ repeat disease (Apostol
et al., 2003). Prevention of aggregation of polyQ molecules in Drosophila
model has been investigated (Sang et al., 2005). Components of endocytic
complex (EC), Sla1, Sla2 and Pan1 observed (Merin et al., 2003) as clusters
at the late stage of maturation of the polyQ monomer or soluble oligomers
participate in aggregation. An ubiquitin ligase, called E-6-AP, which is able to
promote the proteasomal degradation of misfolded polyQ proteins and
suppress the polyQ protein–induced cell death, has been identified (Mishra et
al., 2008). There is a report that polyQ aggregates may not be toxic but
extended polyQ stretch alters the binding of htt to other cell proteins
(Khoshnan et al., 2002).

Protein transduction domains (PTDs)-mediated *in vivo* delivery of aggregate
inhibitors are a general strategy for the treatment of neurodegenerative
diseases involving abnormal aggregation of misfolded proteins (Popiel et al.,
2007). Cell therapy strategies to protect vulnerable neuronal cell population or
to replace dysfunctional or dying cells have also been carried out (Clelland et
al., 2008) through the manipulation of endogenous stem cells and/or
neurogenesis to protect susceptible neuronal populations.
Among various stages of therapeutic strategies for polyQ disease reviewed (Shao and Diamond, 2007), direct targeting of polyQ aggregation has been a focus of therapeutic development for several years. Attempts have also been made on screening the small molecules that directly interfere with polyQ protein aggregation. On the other hand some relevant data in cell-based assays and model organisms few convincing results in mice have been described. Being so, the compounds designed to prevent formation of large aggregates may not stop the initial pathological misfolding of protein monomers, which will retain their capacity for pathogenesis, either as single molecules as or as toxic oligomers. In the review (Rubinsztein, 2006), it has been stated that dysfunction of the ubiquitin-proteasome or enhancing macroautophagy with drugs such as rapamycin may offer a tractable therapeutic strategy.

After having analysed the four brain regions, caudate nucleus, cerebellum, prefrontal association cortex and motor cortex, of 44 human HD brains the findings has suggested that differential gene expression in HD brain shows a distinct regional pattern that generally parallels the known pattern of neuronal loss. The ubiquitous distribution of huntingtin in the somatic cytoplasm of developing mouse brain neurons suggests that the protein has a constitutive role important for neuron survival (Bhide et al., 1996). As the gene expression profiles of HD caudate and HD motor cortex are strikingly similar, there may be similar general molecular characteristics to the neurodegenerative process indifferent regions of the brain (Hodges et al., 2006). It has been demonstrated that the HD-associated CAG repeat stretch in neurons is capable of changing its length over 300 CAG repeats (Shelbourne et al., 2007) to the extremely large alleles (> 500 CAG repeats) in investigations of human HD brain material (Kennedy et al., 2003).

Normal huntingtin is co-localised with post-synaptic density 95 (PSD95), and expression of the mutated huntingtin cause translocation of the scaffold protein and inhibits the co-localization of these two proteins in the cytoplasm (Song et al., 2003). PolyQ-GFP (Green Fluorescence Protein) expression is toxic to primary neurons but that the cell death is distinct from classical apoptosis (Moulder et al., 1999). Caspases, cysteine proteases responsible
for the terminal cleavage events in apoptosis, were activated in granule cells in response to polyQ aggregation by Q80-GFP but inhibition of caspasse activation did not block polyQ toxicity. Cerebellar granule neurons may provide a model system in which to test potential therapies for polyQ toxicity and for the pathogenic mechanisms in these diseases.

It is clear from the conformational studies and polyglutamine peptides by various experimental and simulation studies that the conformational results are at variance. Even, it is supported by experimental by at the genetic level. There is hardly any report on the effect of the solvent on the conformational behaviour and aggregation. In polyglutamine peptides, there is amide linkage both in the backbone as well as in the side chain. Hardly, any attention has been paid on this aspect, it is because of these things this study deals with conformational behaviour of polyglutamine peptides and their aggregation behaviour of varying chain length. Does the polyglutamine peptide adopt a regular secondary structure or not? What are the stabilising interactions in the monomeric form of glutamic peptides? This studies through light on the aggregation behaviour of polyglutamine peptides which may help some insights in understanding the polyglutamine diseases. But certainly, one must be cautious in interpreting the results as the structure of polyglutamine stretch in the presence of the huntingtin protein may be different. This can also alter the aggregation behaviour.