Proteinopathies: A review on current scenario and therapeutic interventions

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Abstract

There are certain neurodegenerative as well as non-neurodegenerative disorders whose characteristic feature is the presence of protein aggregates. The aggregates can be of different types, shapes, and sizes. These aggregates are harmful because they sequester the proteins and RNAs essential for cellular function. Protein aggregates found in these proteinopathies are thus an area of major interest in the scientific community. In recent years, scientists have tried to develop diagnostic and therapeutic measures targeting the protein aggregates. This review article aims to provide an overview of the advances that have been made in the last five years in terms of understanding of the protein aggregates found in the disease, its diagnosis, and its treatment. To achieve this, PubMed and Google Scholar were searched for reviews and research papers published from 2015-2020 using the following keywords: 'protein aggregation', 'proteinopathy', 'protein aggregation disorders' etc.

Keywords: Protein aggregation, Proteinopathy, Protein aggregation disorders.

1. Introduction

Proteinopathies are a class of disorders in which proteins are found in an aggregated form either intracellularly or extracellularly. The proteins found in such aggregates are either not able to perform their normal function (loss-of-function) or they perform a function not required by the cell (gain-of-function) (Luheshi et al. 2008). The disorders containing such aggregates can range from Alzheimer's, Parkinson's, Huntington's to Type 2 diabetes, Amyloidosis, Cystic Fibrosis, etc. Mostly the proteinopathies arise sporadically, in some cases, they are inherited, and in very rare cases they could be a result of the transmission of infectious agents (as in Prions disease) (Moreno-Gonzalez and Soto 2011).

Protein aggregates are a hallmark feature of proteinopathies. The different protein aggregates like amorphous deposits, amyloid fibrils, native-like assemblies are formed by aggregation of unfolded, partially folded, and fully folded proteins respectively (Chiti and Dobson 2017). Amyloids are the highly ordered, well-defined fibrillar aggregates with cross-beta structures that produce a red-green birefringence upon staining with Congo Red (Aguzzi and O'connor 2010). Protein aggregates are very harmful to the cell as they sequester other cellular proteins and RNA to form inclusion bodies. Scientists have identified various reasons to account for aggregate formation. Aggregates could be formed either because of environmental factors like age, stress, or because of alterations in genes encoding for the disease-associated protein (Ross and Poirier 2005).

Different proteins are accumulated in different proteinopathies (see **Table 1**). The different disease-associated proteins do not have a similar sequence, structure, or function (Chiti and Dobson 2017). However, the mechanism of protein misfolding and aggregation, its intermediates, and the end-product are similar. Many models have been suggested to account for the protein misfolding and aggregation. Among them, the seed-nucleation model given by Lansbury et al is well accepted (Soto and Pritzkow 2018). According to this model, aggregation takes place in two stages: nucleation phase and elongation phase **(Fig. 1)**.

Nucleation phase is a phase in which slowly over time a seed or a nucleus (polymerized protein structure) is formed. Then comes the elongation phase in which seeds quickly grow into aggregate by adding monomers to themselves. Seeds are very harmful to the cell as they can even normal, soluble proteins to form aggregates (Soto and Pritzkow 2018).

Even though protein aggregates and inclusion bodies are a hallmark of proteinopathies there is a controversy surrounding their role in the development of a disease. When various scientists injected the disease-associated protein into mouse models they observed manifestations similar to those seen in humans so they presumed that aggregates and inclusion bodies played a role in pathogenesis (Ross and Poirier 2005). However, a discrepancy was seen when scientists observed that cells with inclusion bodies were not the ones that were dying. Scientists induced mutations in the mouse models to prevent inclusion body formation, and this resulted in increased cytotoxicity (Takeuchi and Nagai 2017). The scientists thus concluded that inclusion bodies are not harmful to the cell but the intermediates of the inclusion body formation process are the ones that are toxic.

To prevent protein aggregation cells, have various defense mechanisms in place. Molecular chaperones, proteasome degradation, and autophagy are the mechanisms that cells use to prevent aggregation. With increasing age, these defense systems start declining and thus leading to aggregate formation (Hartl 2017). The aim of this review is to briefly outline the main aspects of various proteinopathies and the various advances that have been made in recent years to diagnose and treat such disorders.

2. Neurodegenerative Disorders

2.1 Alzheimer's disease (AD)

It is a neurodegenerative disorder in which there is a gradual loss of memory, speech, recognition of people and objects, and task performance (Jouanne et al. 2017). The hallmark feature is the presence of two kinds of protein aggregates in the brain, senile plaques and neurofibrillary tangles (NFTs). Senile plaques are composed of amyloid-beta protein and are

found extracellularly whereas neurofibrillary tangles are composed of hyperphosphorylated, microtubule-associated tau protein and are found intracellularly (Majd et al. 2015).

AD (familial form) arises because of mutation either in the gene encoding for amyloid precursor protein (APP) or in the genes encoding for proteins involved in APP cleavage, PSEN1 (presenilin 1) & PSEN2 (presenilin 2) (Lane et al. 2018). And, on the basis of time of onset of symptoms, AD can be classified into-: early-onset AD (EOAD) in which symptoms can be seen pretty early (before 65 years of age) and late-onset AD (LOAD) in which the symptoms are visible after 65 years of age (Chiaravalloti et al. 2016). There are invasive and non-invasive methods to diagnose AD. Invasive methods include analysis of cerebrospinal fluid (CSF) or serum assays whereas non-invasive methods include imaging (Weller and Budson 2018). Functional and structural imaging methods like PET (Positron Emission Tomography), SPECT (Single-photon Emission Computed Tomography), and MRI (Magnetic Resonance Imaging) are used to detect beta-amyloid and tau protein aggregates (Jouanne et al. 2017). Imaging involves the detection of protein aggregates but this aggregation is seen late in AD, thus for early diagnosis, CSF level is checked for amyloid-beta peptide and tau protein (Jouanne et al. 2017). Serum assays, a less-invasive diagnostic method detects the number of circulating disease-associated proteins (Weller and Budson 2018).

No cure for AD is currently known. Conventional therapy (involving the use of drugs) or modern therapies are used to treat AD. There are two classes of drugs that can be used for the treatment of AD: cholinesterase inhibitors (eg; Galantamine, Rivastigmine, Donepezil) and NMDA (N-methyl-d-aspartate) receptor antagonist (eg; Memantine) (Kumar and Singh 2015). For patients with moderate-to-severe AD, memantine is used whereas cholinesterase inhibitors can be used for any stage of AD. These drugs slow down the development of a disease and help with the symptoms of the disease but they do not provide a cure. Modern therapy involves the use of monoclonal antibodies to remove the amyloid-beta aggregates in

the brain **(Fig. 2)**. Drugs targeting the enzyme involved in in the cleavage of APP and anti-tau drugs are being made **(Fig. 2)** (Weller and Budson 2018).

2.2 Parkinson's disease

Subsequently AD, this is the most common neurodegenerative disorder that affects older people (> 60 years of age). In this disorder, neurons present in part of the brain responsible for movement are degenerated resulting in impairment of the motor functions of the affected individuals (Majd et al. 2015). PD is two times more prevalent in men than in women (Radhakrishnan and Goyal 2018). Clinical manifestation of PD includes the appearance of motor and non-motor symptoms. Motor symptoms include tremor, rigidity, bradykinesia whereas non-motor symptoms include sleep disorders, gastrointestinal dysfunction, hallucinations (Reich and Savitt 2019). Non-motor symptoms manifest about 10 years earlier than the motor symptoms. Mostly, the symptoms start on one side of the body and appear on the contralateral side within a few years (Sveinbjornsdottir 2016).

The hallmark feature of PD is the presence of two kinds of protein aggregates in the cytoplasm of the cells namely Lewy bodies (LB) which are spherical shaped and Lewy neurites (LN) which are thread-like aggregates (Sveinbjornsdottir 2016). Mainly, these aggregates are found in the dopamine-releasing neurons present in substantia nigra and the main component of these protein aggregates is the alpha-synuclein protein (Majd et al. 2015).

Diagnosis involves a physical examination of the patient in which patients are checked for motor symptoms like bradykinesia (slow movement), rigidity, resting tremor, imbalance, etc. Moreover, affected individuals also checked for non-motor symptoms like rapid eye movement, sleep disorder, hallucinations. Other strategies that are being used by doctors for a definite diagnosis of PD are DaTscan, and response to dopaminergic therapy (Reich and Savitt 2019).

No effective treatment has yet been developed. Physical and pharmacological therapy is commonly used to manage symptoms (Reich and Savitt 2019). Commonly used medicine for PD is Levodopa which works by decreasing dopamine levels or mimicking its effect at the dopamine receptor (Radhakrishnan and Goyal 2018). These strategies do not work for every PD patient especially not for the patients with severe PD. To treat such cases, interventional therapies like Duopa, Deep Brain Stimulation are used (Reich and Savitt 2019).

2.3 Huntington's disease (HD)

It is a progressive, autosomal dominant neurodegenerative disorder in which a person's motor, cognitive, and behavioral functions are disrupted (Wyant et al. 2017). In this genetic disorder, aggregates, or inclusion bodies mainly composed of huntingtin protein are found in the nucleus leading to the death of the neuron (Illarioshkin et al. 2018). Protein aggregates are found in the striatum and cerebral cortex regions of the brain leading to their neurodegeneration (Cepeda and Tong 2018). This disorder is caused by the expansion in the number of CAG (which encodes for glutamine, Q) repeats present in the Huntingtin gene (Cepeda and Tong 2018). If the number of repeats present exceeds 40, the disease will develop (Wyant et al. 2017). Studies have shown that the number of repeats and age of onset of disease have a negative correlation i.e. longer the repeat, earlier the onset (Chen and Wolynes 2017).

Huntingtin is a 3100 amino acid long protein that is required for proper brain development. It contains a 17-residue sequence at N-terminal (NT17), a polyQ stretch, and a proline-rich region at C-terminal. Mutant Huntingtin gene produces a faulty Huntingtin protein with an expanded polyQ stretch. Such a protein has a propensity to misfold and form beta-sheet fibrillar structures (Chen and Wolynes 2017). Other than HTT protein, inclusion bodies contain proteins like chaperones, transcription factors, cytoskeletal, and proteasomes (Takeuchi and Nagai 2017).

Diagnosis is mostly done by looking for any of the three symptoms associated with HD along with the family history (Roos 2010). Genetic testing is done to verify the cause of manifestations. Other methods like blood testing and imaging are not required for diagnosis. In some cases, the option of pre-manifestation/pre-symptomatic, prenatal, and pre-implantation diagnosis by genetic testing is available (Roos 2010). Research is being carried out to identify biomarkers that can be used to diagnose the disease. Studies have shown that imaging techniques like MRI and PET can be used to predict the age of disease onset, to understand disease evolution, and to recognize molecular changes taking place in the brain as the disease progresses (Ciarmiello et al. 2017).

To treat HD, there are two approaches that scientists are using to prevent protein aggregation. First, small compounds called inhibitors are being made which bind to the expanded polyQ segment and suppress its aggregation; second, activation of the cell's protein quality control system (Takeuchi and Nagai 2017). Short peptides like QBP1 (polyQ binding peptide 1), single-chain antibodies (intrabodies), and antibody 1C2 have been made which have successfully prevented aggregation and cytotoxicity in animal models (Takeuchi and Nagai 2017). Chemicals like Congo red, benzothiazole, and ECGG (epigallocatechin gallate) have also shown great results (Takeuchi and Nagai 2017). By overexpressing chaperones, and using compounds like trehalose and rapamycin to activate autophagy and proteasome degradation scientists have been able to inhibit protein aggregation and improve disease phenotypes **(Fig. 2)** (Takeuchi and Nagai 2017). Gene silencing strategies like RNAi and miRNA are being developed to suppress transcription of the mutant Huntingtin gene **(Fig. 2)** which resulted in improved disease phenotype, and reduced aggregation/inclusion (Takeuchi and Nagai 2017). No permanent cure for the disease exists as of yet.

2.4 Amyotrophic lateral sclerosis (ALS)

ALS is a neurodegenerative disorder characterized by progressive degeneration of motor neurons and other neurons found in the brain and spinal cord. This leads to the appearance of

heterogeneous presentations like wasting, cramps, clumsiness, fasciculations, spasticity, etc (Oskarsson et al. 2018). Some patients can even have difficulty with speech or swallowing, and may cry or laugh uncontrollably (pseudobulbar affect) (Morgan and Orrell 2016; van Es et al. 2017). Problems with respiration lead to the death of ALS patients which usually occurs within 2-3 years from symptom onset (Morgan and Orrell 2016; Rossi et al. 2018). The most common cause of ALS is the genetic mutation in genes like C9orf72, FUS, SOD1, TARDBP, and 30 other genes (van Es et al. 2017). Environmental factors like smoking, athletic disposition, head injury, exposure to metals, and pesticides may be responsible for ALS but as of yet no definite proof as such exists (Oskarsson et al. 2018; Riva et al. 2016). Some scientists believe that the reactivation of the viral genes present in the human genome may cause ALS (van Es et al. 2017).

The characteristic feature of ALS is the presence of ubiquitinated, phosphorylated, and truncated cytoplasmic aggregates of TDP-43 in the neuron (Feneberg et al. 2018). These aggregates are seen in almost all patients except for the patients with SOD1 and FUS mutations (van Es et al. 2017). TDP-43 (TAR DNA-binding protein 43) is a 414 amino acid long RNA binding protein that regulates processes like transcription, mRNA processing, etc (Morgan and Orrell 2016). Under normal conditions, TDP-43 is localized to the nucleus but in ALS patients it is found in cytoplasmic inclusions (Feneberg et al. 2018). Due to its aggregation in the cytoplasm, the level of TDP-43 in the nucleus of motor neurons depletes which results in dysregulation of RNA metabolism and processing (Morgan and Orrell 2016). In patients with C9orf72 mutations, other than TDP-43, dipeptide repeat proteins are also found in the cytoplasmic inclusions (Morgan and Orrell 2016). These proteins are produced by translation of repeat expansions of C9orf72 and they bind to RNA-binding proteins thus disrupting their function (Oskarsson et al. 2018).

Currently, ALS is diagnosed by a clinical examination that involves looking for the extent of upper and lower motor neurons involvement (Riva et al. 2016). In the initial stages of ALS,

because of the heterogeneous range of presentations diagnosis becomes difficult and is delayed (van Es et al. 2017). Because of delayed diagnosis, the majority of motor neurons degenerate in turn making the development of therapies difficult (Paré et al. 2015). The TDP-43 aggregates can't be used for diagnosis as they are found at a pretty late stage in ALS (Paré et al. 2015). Pare et al. have observed in their study that skin changes in ALS patients can be seen before the symptom onset and thus can be used for early diagnosis (Paré et al. 2015). Further research has to be done on the Motor function impairment could be a result of some other disease so differential diagnosis is required to exclude such cases (Oskarsson et al. 2018). Electromyography, neuroimaging, genetic testing can help with the diagnosis of ALS (Oskarsson et al. 2018). Testing CSF for dipeptide repeat proteins can be a possible diagnostic tool (van Es et al. 2017). No standalone diagnostic test is yet available.

Currently, there is no treatment available for ALS. Riluzole and Edaravone are the two medications that are currently used for the pharmacological treatment of ALS (Oskarsson et al. 2018; Riva et al. 2016). Other treatment strategies involve symptomatic care tailored to an individual's need with assisted ventilation (Riva et al. 2016). Modern therapeutic strategies like stem cell therapy and antisense oligonucleotides are currently under development. Stem cell therapy focuses on the use of stem cells to preserve the remaining number of motor neurons (Oskarsson et al. 2018). Additionally, the antisense oligonucleotides targeting SOD1 and C9orf72 are also being developed **(Fig. 2)** (Oskarsson et al. 2018)

2.5 Frontotemporal dementia (FTD) It is a highly heterogeneous group of neurodegenerative disorders characterized by changes in behavior, language impairment, and loss of executive control (Borroni and Benussi 2019). Right after AD, FTD is the second most common form of dementia in people under the age of 65 [32]. The average age of onset is between 45 and 65 with men and women both equally affected (Olney et al. 2017). On the basis of symptoms, FTD can be categorized into three categories namely behavioral variant of FTD (bvFTD), and two primary progressive aphasias (PPA): non-fluent variant/agrammatic primary progressive

aphasia (nfvPPA) and semantic variant primary progressive aphasia (svPPA) (Tsai and Boxer 2016). bvFTD is characterized by changes in behavior, personality, emotion, and executive control. PPAs are disorders in which language impairment is the primary manifestation and are further categorized into-: svPPA is characterized by progressive loss of semantic knowledge and behavioral changes, and nfvPPA is characterized by slow, choppy, effortful speech (Olney et al. 2017).

Primarily, tau and TDP-43 (TAR DNA-binding protein 43) proteins are found in the intracellular aggregates. Though less common but FET family proteins and unknown ubiquitinated proteins are also found in the aggregates (Sivasathiaseelan et al. 2019). Thus, on the basis of the protein that gets deposited, FTD can be subclassified into the following: FTD-TDP-43, FTD-tau, FTD-FET, and FTD-UPS (Olney et al. 2017). Scientists have identified several genetic mutations to account for the aggregation of such protein in FTD. The most common mutations are those in the MAPT (microtubule-associated protein tau) gene, the GRN (granulin) gene, or the expansion in C9orf72 (chromosome 9 open reading frame 72) gene (Borroni and Benussi 2019). Different mutations are responsible for different FTD subtypes. For example, the GRN and C9orf72 mutations correspond to FTD-TDP whereas MAPT mutations correspond to FTD-tau (Borroni and Benussi 2019).

According to several reports, a family history check and neuroimaging (PET/MRI) with a proper examination are mandatory for a good diagnosis (Borroni and Benussi 2019; Olney et al. 2017; Sivasathiaseelan et al. 2019). According to Hu et al., to diagnose FTD-TDP, the patient's ratio of phosphorylated tau to total tau in cerebrospinal fluid is checked, with a low ratio indicating FTD-TDP (Borroni and Benussi 2019). For pre-symptomatic analysis, plasma and CSF are checked for polyG and GRN levels (Borroni and Benussi 2019). Another technique that can be used for pre-symptomatic analysis is the use of transcranial magnetic stimulation (Borroni and Benussi 2019).

Currently, there is no approved treatment for FTD but off-label drugs and non-pharmacological therapy can be used to manage the symptoms (Olney et al. 2017). In recent years, understanding regarding FTD has increased which has given way for the development of novel therapeutic strategies. To remove tau aggregates, scientists are developing anti-tau antibodies **(Fig. 2)** (Sivasathiaseelan et al. 2019). Wischik et al. made a compound called LMTx (Leuco-methylthioninium) which inhibits tau aggregation by blocking an important process required by tau to form filaments. Currently, it is under trial for FTD (Tsai and Boxer 2016). Another strategy that scientists are developing agents that can prevent phosphorylation of tau by either inhibiting activity of protein kinase or by enhancing phosphate activity (Tsai and Boxer 2016). This is believed to help prevent tau protein aggregation. To make up for the reduced levels of granulin in CSF and serum, scientists have theorized that agents that can elevate its level can be made (Tsai and Boxer 2016). In fact, several such agents are currently under trial. Antisense oligonucleotides targeting toxic C9orf72 mRNAs are currently being developed for FTD treatment **(Fig. 2)** (Sivasathiaseelan et al. 2019).

2.6 Prions disease

Prions disease or transmissible spongiform encephalopathies (TSEs) are a group of diseases linked to neuron degeneration in both humans as well as in animals. The first prion disease found was the scrapie in sheep and goats in the 1730s (Collinge 2016). Whereas, The first prion disease identified in 1920 in humans by Creutzfeldt and Jacob and these are now known as the CJD and are divided into three major classes namely sporadic, inherited, or acquired (Baldwin and Correll 2019; Tee et al. 2018). The deposition of these prions' protein occurs in a wide range and can vary from minute deposits and uneven distribution of prions in synaptic clefts to the deposition in the neurons as patches of amyloid protein. Further, in recent years more of these diseases' variants have been identified in humans and animals, one of the most common among them is bovine spongiform encephalopathy. These prion

proteins, once formed in the body, recruit other prions at the site of infection and also transmit the toxicity to the normal protein present in the body as well.

Prions disease is associated with the misfolded prions protein devoid of any nucleic acid, production, and aggregation in the biological system (Tee et al. 2018). Prion protein (PrP^{sc}) acts as a precursor and modifies the normal protein into a pathogenic form. During the protein synthesis in ER, a 22 amino acid long secretory signal peptide is cleaved from the N terminus of the prion and C terminal signal sequence for the attachment of the glycosylphosphatidylinositol anchor which help in the attachment to the membrane and laterally in the deposition. The N terminal of this protein is flexible and contains an octameric repeat region, neurotoxic domain, and a hydrophobic core (Bernardi and Bruni 2019). Different variants of prion diseases are characterised on the basis of methionine/valine substitution at codon 129 of the PrP gene (PRNP) (Gambetti et al. 2011).

Diagnosis of prion disease mostly depends upon behavioral symptoms determination and almost everyone with dementia is diagnosed with prion disease. Clinical diagnosis of the disease is confirmed with an electroencephalogram, magnetic resonance imaging (MRI), cerebrospinal fluid for tau proteins, and PrP-amyloid aggregation assays with recently developed real-time PCR tools (Zerr and Parchi 2018).

There is no effective treatment available for the prion disease and their subtypes however, doctors majorly prescribe medicine to counter the pain and symptoms of the disease. With the recent advancement and understanding of the molecular pathogenesis of the disease, various compounds and protocols have been explored by the scientist in the development of therapy for CJD including chemical therapy, RNAi mediated therapies, antibodies-based therapy, and natural compound-based therapy (Zafar et al. 2019).

3. Non -Neurodegenerative disorders

3.1 Type 2 diabetes (T2D)

Type 2 diabetes (T2D) is a systemic metabolic disease found to be associated with the destruction of beta-cells (β cells) in the islets of Langerhans. T2D is one of the most common diseases found in humans, more frequently at the later stages of their lives (>45 years). The major factors contributing to developments of T2D involve obesity and dispositioning of genetic factors as well as hereditary transfer. β cells function in secretion of insulin as well as human islet amyloid polypeptide (hIAPP) upon stimulation with sugar molecules. hIAPP (also known as amylin) deposition is correlated with the onset of insulin resistance and the development of T2D in humans (Pang et al. 2020). Epidemiologically >90% of T2D patients have been diagnosed with the increased lesion of hIAPP in pancreatic β cells (Mukherjee and Soto 2017).

Amylin is secreted as a 37 amino acid long polypeptide with an amyloid prone sequence in between 20-29, single serine to glycine (S20G) mutation results in forming more aggressive amyloid aggregates (Meier et al. 2016). Aggregated forms of hIAPP have different size and shapes such as oligomers, protofibrils, fibrils, and amyloids out of which the hexamer oligos are most toxic and frequently form amyloid aggregates (Lao et al. 2019). Deposition of amylin or hIAPP results in the formation of reactive oxygen species (ROS) which in turn results in increased apoptosis and loss of pancreatic β cells (Rehman and Akash 2017).

Diagnosis of T2D involves the observation of the symptoms related to the onset of the disease such as frequent thirst and urination and later confirmed with a simple blood test to quantify blood sugar level. In recent years, imaging probes have been developed to target Amylin aggregates in T2D as well (Templin et al. 2018).

The treatment procedure for T2D involves the application of oral and injectable antidiabetic drugs such as metformin along with mimetics and amyloid formation inhibitors [48]. Other than this various methods such as the use of natural inhibitors, oligopeptides inducers of

autophagy **(Fig. 2)**, etc. are being explored as therapy options and are currently under clinical trials and developmental stages (Lao et al. 2019; Zhang et al. 2019).

3.2 Cystic fibrosis (CF)

Cystic fibrosis (CF) is a class of heterogeneous multi organ characterised with malfunction of the CFTR gene and thereby forming a dysfunctional misfolded protein product (Krainer et al. 2020). Mutations in the CFTR gene are related to the severity of diseases to many different organs of which CF associated with the reduced function of lungs due to the accumulation of misfolded proteins have been identified as the most severe form. Other organs such as the digestive system, reproductive system are also common and require lifelong medical intervention and therapies in order to reduce symptoms and counter disease progression (Fraser-Pitt and O'Neil 2015).

Cystic fibrosis transmembrane conductance regulator (CFTR) ion channel protein is a transmembrane chloride ion channel that becomes defective in the case of CF. It is an autosomal recessive disease which arises due to mutation in the CFTR gene resulting in the formation of non-functional or dysfunctional ion channels (Fraser-Pitt and O'Neil 2015). The three classes of mutation are majorly associated with the onset of disease and are characterized by the different amino acid sequence alterations. The first type is defined with the change in the guanine residue (G) at 542 position to Thymine (GGA-TGA) (Fraser-Pitt and O'Neil 2015). In the second class which is also the most common type of CF i.e. F508del-CFTR, or delta-F508 (ΔF508), characterized by the deletion of phenylalanine at 508 position in the polypeptide chain (Hutt et al. 2018). Furthermore, Class three CF mutation involves a missense type of mutation such as mutation of glycine (G) at 551 position into aspartate (D) (G551D) (Harris et al. 2020). There are other variants and classes that are also available in literature such as V232D, E217G, etc (Krainer et al. 2020; Krainer et al. 2018; Strug et al. 2018).

Diagnosis of CF initially involves the identification of the symptoms and Phenotypic history with diseases among siblings. With the increase in asymptomatic patients found in newborns, screening of newborns with the help of confirmatory sweat chloride test is required to identify the new cases. Whereas, when a patient has moderate chloride concentration in their sweat CFTR genetic analysis is performed for the confirmation of the CF (Farrell et al. 2017).

There is no permanent cure available for Cystic fibrosis however based on the symptoms, disease etiology, health condition, and age, a number of medical interventions are applied to reduce the symptoms of the disease (Fraser-Pitt and O'Neil 2015). Recently, with the help of improved molecular technology and gene modification systems **(Fig. 2)**, the treatment procedure is now more concentrated on correcting the defective gene with targeted gene therapy and gene editing protocols (Strug et al. 2018).

3.3 Cataract

Cataract, or opacification of human lenses results in blindness which is associated with the accumulation of lens proteins inside the lens environment. These lens proteins or Crystallin family of proteins constitute 90% of total lens protein and are responsible for the transparency of the eye lens where these crystallin proteins can resist protein aggregates formation (Moreau and King 2012). Aggregation of these crystallins protein either due to mutation, damage, post translational modification, or aging could result in cataract formation (Andley et al. 2018). There are α, β and γ-crystallin families which are water-soluble of which, α-Crystallin is a molecular chaperone which prevents the accumulation of aggregates at the lens (Andley et al. 2018). When malfunctioning proteins overwhelm the available heat shock proteins formed due to photodamage, oxidation, deamidation, etc results in protein accumulation and scattering of light causing cataract.

Crystallins are highly expressed proteins in mammalian eye lenses and constitute three major groups α, β, and γ-crystallin (Graw 2009). These proteins are highly stable and do not show

any turnover and help in maintaining a clear environment around the lens and hence a clear vision and refractive index of the eye. Impairment of the stability of these proteins is majorly associated with mutation, environmental stress, and various chemical modification including oxidation and deamidation (Moreau and King 2012; Ramkumar et al. 2018). Deamidation is the most common type of modification involving negative charge transfer to the protein by converting glutamine to glutamate. Other than deamidation several tryptophan present at the core and cysteine residues of β and γ-crystallin are subjected to oxidation and are mostly found in aged lenses. Furthermore, many mutations such as G64W, S39C, etc cause susceptibility to stress or can also regulate gene expression of crystallins protein (Graw 2009; Li et al. 2019; Ramkumar et al. 2018; Yang et al. 2020). The above-mentioned mutation of these molecular chaperones hinders their ability of protein sorting and folding which results in formation of proteins aggregates at the site of the lens causing loss or reduced vision.

To determine if a patient is having cataracts or not, doctors review the medical history for associated risk factors and general symptoms of the disease. For further confirmation, the doctor performs several eye tests including a retinal exam, a virtual acuity test, and a slit-lamp test. Genetic identification of mutants has been explored in recent years to identify the hereditary form of cataract in neonatal and children (Zanolli et al. 2020).

There is no medicinal prescription available for the complete cure of cataract and patients are advised to wear ocular glasses to maintain clear vision. However, when these prescription glasses cannot clear your vision only effective treatment available is the surgical replacement of the lens having aggregated proteins with an intraocular lens (Liu et al. 2017). Postoperative management is essential for the recovery and improved maintenance of the artificial lens.

4. Conclusion

Proteinopathies are a class of diseases characterized by the formation of abnormality in certain proteins arising due to genetic changes causing them to form protein aggregates.

These aggregated forms of the protein show cytotoxic effects on the cells thereby the preferred diagnostics uses the screening of these protein aggregates. Screening procedure including CSF analysis, genetic testing, imaging, etc. helps in identifying these aggregates however this approach offers only a limited advantage as far as diagnosis is concerned as protein aggregates are generally observed at the later stages of the disease. Furthermore, the currently available options for therapeutics focus only on providing symptomatic care or temporary relief. In the last decade, the focus has shifted to the improvement of therapeutics by targeting the protein aggregates in two ways: either inhibiting the formation of aggregates or by removing the protein aggregates to reduce their cytotoxic effects. Thus, moving forward, the major focus of future studies should be more concerned with the development of the diagnostic techniques for the assessment of the disease in their early-stage along with the assessment of the efficacy of modern therapeutic measures that are currently under development in providing relief to the patients.

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Table

Table 1 Various proteinopathies and the protein(s) that is/are found in their aggregates

Neurodegenerative (ND) or Non-Neurodegerative (NND)	Proteinopathy	Disease associated protein (s)	Associated Gene	Mutation	Refrences
ND	Alzheimer's Disease (AD)	Amyloid- β & Tau	APP (Amyloid Precursor Protein), PSEN 1 (Presenilin 1), PSEN 2 (Presenilin 2), APOE (Apolipoprotein E)	Substitution at codon717 (Val-Ile); L166P; N141I	(Bekris et al. 2010; Lane et al. 2018)
	Parkinson's Disease (PD)	α-synuclein	SNCA (Synuclein Alpha) gene, LRRK 2 (Leucine-rich repeat kinase 2), VPS35 (Vacuolar protein sorting-associated protein 35), PINK1, PRKN (Parkin)	A53T, A30P; G2019S, R1441G; D620N	(Ferreira and Massano 2017; Ran and Belin 2014)
	Huntington's Disease (HD)	Huntingtin	HTT gene	CAG expansion	(Illarioshkin et al. 2018; Roos 2010; Wyant et al. 2017)
	Amyotrophic Lateral Sclerosis (ALS)	SOD (Superoxide Dismutase); TDP-43 (TAR-DNA binding protein 43); FUS (Fused in Sarcoma)	C9orf72 (chromosome 9 open reading frame 72); SOD1, TARDBP, FUS	Hexanucleotide repeat (GGGGCC) expansion; D90A, A4V; A382T; R521C, R521H	(Lattante et al. 2013; Mejzini et al. 2019)
	Frontotemporal Dementia (FTD)	TDP-43, Tau, FET family proteins	C9orf72, GRN (Granulin), MAPT (microtubule-associated protein tau),	G_4C_2 expansion; A9D; P301L	(Pottier et al. 2016)
	Prions Disease	Prion protein (PrP)	PrP gene (PRNP)	M129V	(Gambetti et al. 2011)
NND	Type 2 Diabetes (T2D)	Amylin (or human islet amyloid polypeptide, hIAPP)		SG20	(Meier et al. 2016)
	Cystic Fibrosis (CF)	CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) protein	CFTR gene	G520T (GGA-TGA); ΔF508 (deletion of Phe); G551D	(Fraser-Pitt and O'Neil 2015; Harris et al. 2020; Hutt et al. 2018)
	Cataract	Crystallin	α, β, γ crystallin gene	Deamidation of Gln to Glu;Oxidation of Trp and Cys;G64W, S39C etc.	(Li et al. 2019; Moreau and King 2012; Ramkumar et al. 2018; Yang et al. 2020)

Figure legends

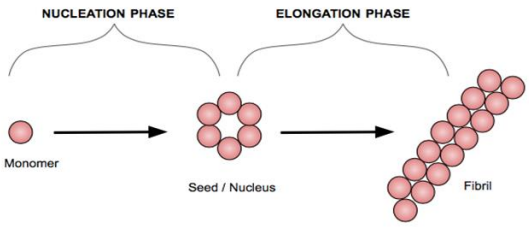

Fig. 1 The seed-nucleation model showing protein aggregate formation

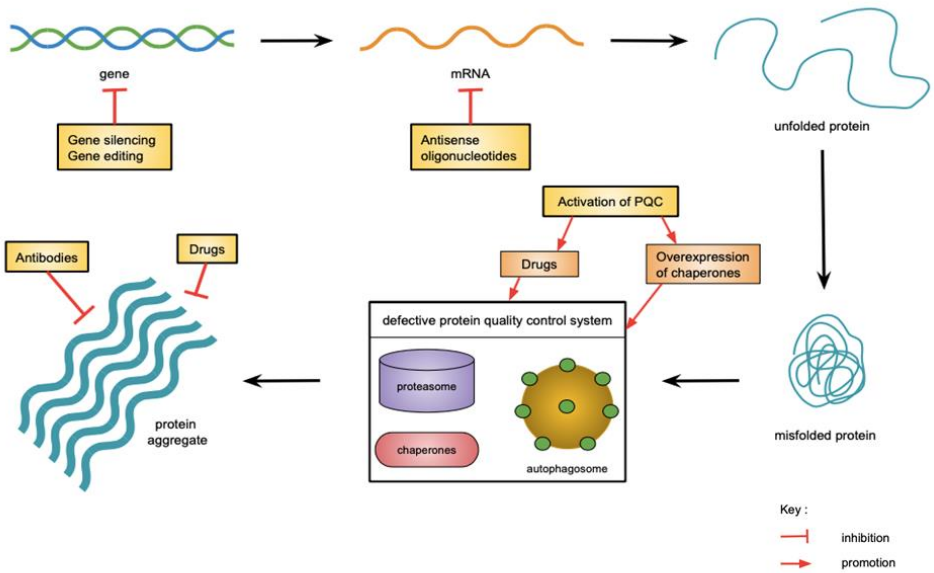

Fig. 2 Various therapeutic strategies currently under development or use to prevent aggregation or to remove protein aggregates