PRION PROTEIN AS A PATHOGEN: A REVIEW

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Abstract
Prion proteins (PrP) are associated with transmissible spongiform encephalopathies (TSE), which are invariably fatal diseases characterized by loss of motor control, dementia, and paralysis wasting. The "protein-only" hypothesis proposes that TSEs are caused by the conversion of a ubiquitous "cellular form" of PrP (PrP^C) into an aggregated "scrapie form" (PrP^{Sc}). According to this model, the prion protein (PrP) would at the same time be target and infectious agent in TSEs, which could explain that this class of diseases can be traced to infectious, inherited, and spontaneous origins. PrP^{Sc} is characterized by high β-sheet content whereas PrP^C is a soluble protein with a high content of α-helices and high susceptibility to proteolytic digestion. Human TSEs include Creutzfeldt-Jakob disease, fatal familial insomnia, the Gerstmann-Straussler-Scheinker syndrome, and kuru, and there is bovine spongiform encephalopathy in cattle and scrapie in sheep.

Keywords: Prion protein, Transmissible spongiform encephalopathies, cellular form, scrapie form, Creutzfeldt-Jakob disease, fatal familial insomnia, Gerstmann Straussler Scheinker syndrome, and kuru

1. Introduction:

Prion is an acronym derived from the words Proteinaceous infection partical[1]. Prion protein is a normal constituent of brain tissue in all mammals [2]. Infectious prion proteins (PrPsc) are not viruses, bacteria[3] because they related to genetics reproduction and propagation [2]. Prion diseases are fatal neurodegenerative disorder known as Transmissible Spongiform Encephalopathics in mammals including human[4]. Infectious PrP^{sc} protein induces a conformational change on the normal PrP^c protein and abnormal complexes are interalised into neurons, damaging the cells and causing the spongiform encephalopathy [3].

2. Discovery:

An American Biochemist and his collegues in 1982, extracted the infectious material from hamster brain. Prusiner suggested that the infectious agents causing certain degenerative disorders of C.N.S. in animals and more rarely in human, is a small Proteinaceous infectious particle, which he called Prion and the protein believed to be responsible for infection was called "Prion related protein" or PrP. In 1988 Prusiner and his team reported that human prion disease can certainty be inherited, i.e. they could be heritable and communicable [5]. Prusiner was awarded the Nobel prize in 1997 for his efforts[6].

3. Structure:

Human prion related protein PrP, a glycoprotein encoded by the PrP gene in which it last replicated. The gene for the physiological isoform of human PrP is located on the short arm of chromosome 20. PrP exists in two isoforms PrP^c and PrP^{sc}. The polypeptide chain of PrP^c and Prp^{sc} are identical in their chemical composition but different in their three dimensional conformations[3].

3.1. PrP^c: The normal protein structure is belived to consist of a number of flexible coils called alpha helices. The normal protein

1) is called PrP^C (for cellular)
2) is a transmembrane glycoprotein normally found at the surface of certain cells (e.g., neural and hematopoietic stem cells)
3) has its secondary structure dominated by alpha helices (probably 3 of them)
4) is easily soluble
5) is easily digested by proteases
6) Is encoded by a gene designated (in humans) PRNP located on our chromosome 20[6].

PrP^c may act as an acetyl-choline receptor inducer and plays an important role in the transmission of nerve signal [5].

3.2. PrP^{sc}: In the aberrant protein some these helices are stretched out into flat structure called beta strands.

1) is called PrP^{Sc} (for scrapie)

2) has the same amino acid sequence as the normal protein; that is, their primary structures are identical but
3) its secondary structure is dominated by beta conformation
4) is insoluble in all but the strongest solvents
5) is highly resistant to digestion by proteases
6) When PrP^{Sc} comes in contact with PrP^{C}, it converts the PrP^{C} into more of itself (even in the test tube).
7) These molecules bind to each other forming aggregates.
8) It is not yet clear if these aggregates are themselves the cause of the cell damage or are simply a side effect of the underlying disease process [6].

PrP^{sc} with much hydrophobic aminoacyl side chain exposed to solvent[5].

4. Pathogenesis:

Prion disease are charaterised by spongiform changes, astrocytic gliosis and neuronal loss resulting from deposition of protein in stable amyloid fibrils [1]. Spongiform refers to the characteristic appearance of infected brains, which become filled with holes until they resemble sponges under a microscope. Incubation period for prion disease ranging from a few months to forty years[7].

Recently, prion infections have been termed amyloidoses. Serpell and colleagues state, "Amyloidoses are diseases...in which soluble proteins are deposited in a specific, highly stable, fibrillar form". Amyloid fibrils have three diagnostic characteristics: under the electron microscope, the fibrils are straight and unbranched with a smooth surface; amyloid fibrils can be stained with Congo Red and subsequently exhibit an apple-green birefringe; and they have a distinct X-ray defraction pattern, indicative of the beta sheets found in the PrPSc formation [8].

5. Prion Replication:

Scientists believe that the replication of a prion particle occurs almost exactly as the duplication of a virus. The mechanism of replication involves the synthesis of polypeptides in the absence of nucleic acid templates and the post-translational modifications of cellular proteins. A polypeptide is a chain of amino acids, and a nucleic acid template is a group of DNA or RNA molecules that carry information to direct cellular functions. For the prion, replication involves converting conventional proteins into prions. The resulting PrP^{Sc} is a four helix bundle protein with four regions of secondary structure, numbered H1 through H4. Prions replicate by recruiting normal proteins to their cause, "flipping" them into a rogue prion-like shape that can go on to infect other cells and animals. This change initiates a chain reaction, and newly converted prions convert other proteins which they come into contact with on the interior of their respective cell membrane[8].

6. Transmission:

These disease are transmissible from host to host of a single species and sometimes even from one species to another, destroy brain tissue giving it a spongy appearance[6].

7. Resistant:

The accumulation of abnormally protease-resistant prion protein (PrP-res) is common to transmissible spongiform encephalopathies (TSE). The degree of resistance of PrPres can vary depending upon the TSE strain and host species, but the TSE-associated forms of PrPres are considerably more resistant to proteinase K than is the corresponding normal PrP isoform (PrP-sen or PrPc). Besides having enhanced protease-resistance, the various abnormal TSE-associated forms of PrP-res (eg. PrP^{Sc}, PrPCJD and PrPBSE) form insoluble aggregates and have higher beta sheet content than PrP-sen. Many types of evidence implicate PrP-res formation as a central process in TSE pathogenesis and TSE agent replication.

7.1. Mechanism of PrP-res Formation: Seeding solutions of the precursor with pre-existing amyloids fibril fragments can greatly accelerate the polymerization of amyloidogenic proteins. Amyloid polymerization often involves an increase in the beta sheet content of the constituent protein. The similarities between PrP-res and other amyloids suggested that the mechanism of PrP-res formation is like that of other amyloids [9].

7.2. Distribution of Protease-resistant Prion Protein: The neuroanatomic distribution of histologic lesions and the immunohistochemical staining (IHC) pattern of a protease-resistant prion protein (PrPres) in brain and lymphoid tissue of free-ranging mule deer in terminal stages of disease and distribution of PrPres in palatine tonsil[10].

8. Types of Prion Disease:

Prion diseases are often called spongiform encephalopathies because of the post mortem appearance of the brain with large vacuoles in

the cortex and cerebellum. Probably most mammalian species develop these diseases. Specific examples include:

Scrapie: sheep

TME (transmissible mink encephalopathy): mink

CWD (chronic wasting disease): muledeer, elk

BSE (bovine spongiform encephalopathy): cows

Humans are also susceptible to several prion diseases:

CJD: Creutzfeld-Jacob Disease

GSS: Gerstmann-Straussler-Scheinker syndrome

FFI: Fatal familial Insomnia

Kuru

Alpers Syndrome

The incidence of sporadic CJD is about 1 per million per year. GSS occurs at about 2% of the rate of CJD. It is estimated that 1 in 10,000 people are infected with CJD at the time of death

Humans might be infected by prions in 2 ways:

1) Acquired infection (diet and following medical procedures such as surgery, growth hormone injections, corneal transplants) i.e. infectious agent implicated.

2) Apparent hereditary mendelian transmission where it is an autosomal and dominant trait. This is not prima facie consistent with an infectious agent [11].
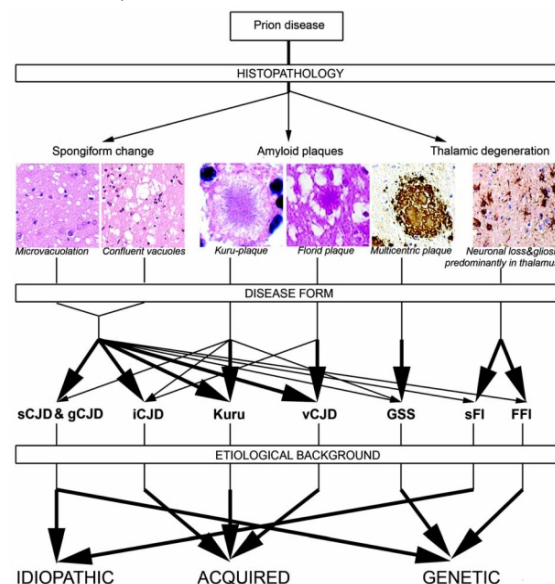

Fig 1: Spectrum of human prion diseases[12]

8.1. Kuru: Kuru is a neurodegenerative disorder. An unknown disease appeared in New Guinea in the early 1900's. By the 1950's anthropologists and government officials reported that this disease termed kuru was rampant among the South Fore. Studies on chimpanzees injected with brain material from a victim led scientists to believe the agent was a slow virus, because the chimps developed a very similar condition after a long incubation period. He defined a slow virus as a viral disease with an abnormally long incubation period. In humans, kuru had an incubation period with a minimum of two years and maximum of 23 years.

8.1.1. Symptoms of Kuru: three main stages in the progression of symptoms.

The first stage is called the **ambulant stage**, and it includes unsteadiness of stance, gait, voice, hands, and eyes; deterioration of speech; tremor; shivering; in- coordination in lower extremities that moves slowly upward; and dysarthria (slurring of speech).

The second stage is also known as the **sedentary stage**, and following symptoms: patient can no longer walk without support, more severe tremors and ataxia (loss of coordination of the muscles), shock-like muscle jerks, emotional lability, outbursts of laughter, depression, and mental slowing (it is important to note that muscle degeneration has not occurred in this stage, and tendon reflexes are usually still normal).

The third stage is the **terminal stage**, which is marked by the patient's inability to sit up without support; more severe ataxia (loss of muscle coordination), tremor, and dysarthria (slurring of speech); urinary and faecal incontinence; difficulty swallowing (dysphagia); and deep ulcerations appear. Cerebellar dysfunction is the cause of these conditions.

Physicians may be able to inject antigene therapies to patients with prion diseases in the future [8].

8.2. Creutzfeldt-Jakob Disease: Creutzfeldt-Jakob disease (CJD) is a rare, degenerative, invariably fatal brain disorder. There are three major categories of CJD:

1) In **sporadic CJD**, the disease appears even though the person has no known risk factors for the disease. This is by far the most common type of CJD and accounts for at least 85 percent of cases.

2) In **hereditary CJD**, the person has a family history of the disease and/or tests positive for a genetic mutation associated with CJD. About 5 to 10 percent of cases of CJD in the United States are hereditary.

3) In **acquired CJD**, the disease is transmitted by exposure to brain or nervous system tissue, usually through certain medical procedures. There is no evidence that CJD is contagious through casual contact with a CJD patient.

CJD belongs to a family of human and animal diseases known as the transmissible spongiform encephalopathies (TSEs). Spongiform refers to the characteristic appearance of infected brains, which become filled with holes until they resemble sponges under a microscope. CJD is the most common of the known human TSEs.

8.2.2. Symptoms: CJD is characterized by rapidly progressive dementia. Initially, individuals experience problems with muscular coordination; personality changes, including impaired memory, judgment, and thinking; and impaired vision. People with the disease also may experience insomnia, depression, or unusual sensations. As the illness progresses, mental impairment becomes severe. Individuals often develop involuntary muscle jerks called myoclonus, and they may go blind. They eventually lose the ability to move and speak and enter a coma. Pneumonia and other infections often occur in these individuals and can lead to death.

There are several known variants of CJD. These variants differ somewhat in the symptoms and course of the disease. For example, a variant form of the disease-called new variant or variant (nv-CJD, v-CJD), described in Great Britain and France-begins primarily with psychiatric symptoms, affects younger individuals than other types of CJD, and has a longer than usual duration from onset of symptoms to death. Another variant, called the panencephalopathic form.

8.2.3. Diagnose: There is currently no single diagnostic test for CJD. When a doctor suspects CJD, the first concern is to rule out treatable forms of dementia such as encephalitis (inflammation of the brain) or chronic meningitis. Standard diagnostic tests include a spinal tap to rule out more common causes of dementia and an electroencephalogram (EEG) to record the brain's electrical pattern, which can be particularly valuable because it shows a specific type of abnormality in CJD. Computerized tomography of the brain can help rule out the possibility that the symptoms result from other problems such as stroke or a brain tumor. Magnetic resonance imaging (MRI) brain scans also can reveal characteristic patterns of brain degeneration that can help diagnose CJD [13].

8.3.   Gerstmann-Straussler-Scheinker: Like Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker disease may occur anywhere in the world. However, it is much less common than Creutzfeldt-Jakob disease, begins earlier in life (affecting people in their 40s rather than in their late 50s and 60s), and progresses more slowly (with an average life expectancy of 5 years rather than 6 months). This disease runs in families [14].

8.3.1. Causes: Since the initial linkage of GSS to a P102L mutation in PRNP,8 GSS mutation (a codon 102 proline to leucine change in PRNP; P102L a variety of mutations in this allele on chromosome 20 have been described in association with this disorder . The most common is the P102L substitution,8,14,15 although other mutations are sometimes relatively prevalent in particular ethnic groups [15].

8.3.2. Symptoms: the first symptoms are clumsiness and unsteadiness when walking. Muscle twitching is much less common than in Creutzfeldt-Jakob disease. Speaking becomes difficult, and dementia develops. Nystagmus (rapid movement of the eyes in one direction, followed by a slower drift back to the original position), blindness, and deafness may develop. Muscle coordination is lost. The muscles may tremble and become stiff. Usually, the muscles that control breathing and coughing are impaired, resulting in a high risk of pneumonia, which is the common cause of death [14].

8.4. Fatal familial insomnia: The fatal familial insomnia disease is of genetic type.

An autosomal dominant disorder characterized by degeneration of the THALAMUS and progressive insomnia. It is caused by a mutation in the prion protein (PRIONS) [16].

8.4.1. Cause of Fatal Familial Insomnia

The cause of fatal familial insomnia is genetic. In more complex term, it's likely due to an autosomal dominant pattern of inheritance. A gene mutation results in the substitution of one amino acid for another. Amino acids are the building blocks of proteins, and this substitution leads to protein misfolding and dysfunction. Ultimately, the protein problems lead to severe loss of neurons and scarring changes called gliosis in part of the brain known as the thalamus [17].

In which inability to sleep during middle age and rapidly degrades into a fatal insomnia.

Today, the fatal familial sleep disorder is so rare that only about 40 families all over the world

have been identified as having the defective gene. If only one of the parents has the gene, the child has a 50% chance of having and developing the disease.

Usually, **fatal familial insomnia's** evolves in four stages. The first stage includes symptoms like lack of sleep that happens suddenly, atypical panic attacks, phobias, which have a total duration of 4 months. The next stage manifests by experiencing lack of sleep and an inability to catch up for the lost hours of sleep. The indications in the first stage worsen as a new symptom takes over; the person starts having hallucinations or false beliefs (either auditory or visual), and this happens for more than 5 months. With the physical degradation, like weight loss happening fast, the patient experiences diminished mental capacity, and the fatal insomnia falls under third stage with the duration of 3 months. The end and 4th stage of the disease is where the patient is already suffering from dementia and the inability to respond to external stimuli with a total duration of approximately 6 months. After these stages patient eventually dies due to the total sleeplessness which slowly progresses into a deep coma.

Currently there is currently no known cure for FFI. This genetic disorder is still covered in mystery, and sleeping capsules only worsen the symptoms. Still, hope is relying in the advancement of a technology called gene therapy. This type of treatment involves the intromission of the correct gene into a sick patient altering his gene structure reverting it to what it should be the construction of the correct protein. This is important because the defective gene needs to be repaired before the disease sets in. For this to be possible, the corrective gene needs to be isolated and must be suitable for transplanting as well as a proper transmitter to effectively accomplish the transfer. Since there is no other cure for this illness, bio-engineering may be the only answer if it is one day successful [18].

Typically the sleep study, or polysomnogram, for fatal familial insomnia will demonstrate an absence of slow-wave sleep. In addition, there will be decreased amounts of stage 2 NREM sleep. Finally, there is dissociated REM sleep without loss of muscle tone. There are few treatment options available for fatal familial insomnia. Medications such as barbiturates and benzodiazepines may be used to induce sleep patterns on EEG. However, the course in fatal familial insomnia is relentless and, as the name implies, ultimately fatal [17].

9. Evidence supports a protein only model of infection:

9.1. Nucleic acid is not necessary for infectivity:
1) Unusually small target size for ultraviolet and ionising radiation:
2) The low ratio of nucleic acid to infectious material.
3) Resistance of infectivity to agents which modify or damage nucleic acids but infectivity is susceptible to reagents which destroy proteins
4) Failure to identify a scrapie specific nucleic acid either in prion preparations or infected brains using a variety of sophisticated techniques.

Table 1: Stabilities of the scrapie agent and viriods (PSTV):

Chemical Treatment	Concentration	PSTV	Scrapie
Et_2PC	10-20mM	(-)	+
NH_2OH	0.1-0.5mM	+	-
Psoralen	10-500µg/ml	+	-
Phenol	Saturated	-	+
SDS	1-10%	-	+
Zn^2+	2mM	+	-
Urea	3-8M	-	+
Alkali	pH 10	(-)	+
KSCN	1M	-	+
Enzymatic Treatment	Concentration	PSTV	Scrapie
RNAse A	0.1-100µg/ml	+	-
DNAse	100µg/ml	-	-
Proteinase K	100µg/ml	-	+
Trypsin	100µg/ml	-	+

+ = inactivated; - = no change in infectivity

9.2. PrP^sc is associated with scrapie infectivity:
1) purification of scrapie infectivity results in preparations highly enriched for PrPsc
2) purification of PrP^sc results in enrichment of scrapie activity
3) purification of PrP^sc by SDS-PAGE also recovers infectivity
4) PrPsc can be denatured and renatured without loss of infectivity

9.3. Susceptibility of a host to prion infection is co-determined by the prion inoculum and the PrP gene:

1) Disease incubation time for a single prion isolate various between mouse strains, this variation depends on the PrP gene, suggesting some forms of PrPc may be more easily converted to PrPsc than others.

2) When prions are transmitted from one species to another disease develops only after a very long incubation period, if at all, but on serial passage in the new species the incubation time often decreases dramatically and then stabilises. This species barrier can be overcome by introducing a PrP transgene from the prion donor i.e. hamster PrPc but not murine PrPc is a suitable substrate for conversion to hamster PrPsc by hamster prions and vice versa:

9.4. Mutated gene can cause susceptibility to disease without apparent infection:

1) Homozygosity at the polymorphic amino acid position 129 of PrP protein predisposes an individual to acquired and sporadic CJD

2) 2 unrelated GSS families have the same double mutation i.e. 178 D-N; 200 E-K[11]

10. Prions in Yeast

Two proteins in yeast (*Saccharomyces cerevisia*) are the Sup35 protein ("Sup35p") and the Ure2 protein (Ure2p), are able to form prions; that is, they can exist either in a PrP^C-like form that is functional or in a PrP^{Sc}-like form that is not.

The greater ease with which yeast can be studied has proved that only protein is involved in prion formation and provided insight into the need for PrP^{Sc} to find PrP^C molecules of a similar primary structure in order to be able to convert them into the PrP^{Sc} form[6].

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