Prions and prion diseases: as a cause of major infections

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ABSTRACT
Proteinaceous infectious proteins known as prions are unique, biological, self-replicating agents linked to a set of rare and fatal neurodegenerative disorders classified as transmissible spongiform encephalopathies (TSEs). TSEs’ self-propagating mechanism is poorly understood besides the fact that prion particles lack nucleic acid makes timely diagnosis and specialized treatment challenging. Presenting a brief historical background, the mechanism for prion infectivity, genetic structure, and possible functions such as cell signaling, cell protection, spatial learning, calcium homeostasis, and copper ion transportation are evaluated. Prions tend to seed and convert from the normal form (PrP\(^c\)) to pathogenic form (PrP\(^\text{sc}\)) leading to a set of serious misfolding diseases. Common human prion diseases include CJD, sCJD, fCJD, vCJD, iCJD, Gerstmann-Straussler-Scheinker Syndrome and Fatal familial insomnia while dominant animal prion diseases include Scrapie, BSE, CWD, EUE and FSE. Prion detection techniques include Western blotting, immunohistochemistry (IHT) and misfolding cyclic amplification method (PMCA). Since prions are extremely difficult and resistant pathogens, specialized sterilization procedures such as autoclaving under extreme conditions and plasma sterilization are recommended.

INTRODUCTION
Proteinaceous infectious particles commonly known as Prions are unique biological, self-replicating agents able to transmit biological information from one organism to another devoid of nucleic acids\(^[1]\). Prions are linked to a set of rare and fatal neurodegenerative disorders classified as prion diseases or transmissible spongiform encephalopathies. The infectious particles create a cluster of holes in the brain similar to a sponge hence the term spongiform encephalopathy. Prion diseases are incurable, sporadic, infectious, and inherited in origin transmitted through misfolding of proteins\(^[2]\). The mechanisms of prion particles are still poorly understood, however, the fact that prion particles lack nucleic acid makes timely diagnosis and specialized treatment challenging. Consequently, prions have longer incubation periods with specific clinical features including debility, ataxia, dementia, anxiety, and hyperesthesia\(^[2]\).

Historically, formal description of prions within the academic and medical circles dates back to 1920s when Jacob and Creutzfeldt identified cases of
progressive motor, neurological and mental deficits in a number of young patients\cite{3}. As a result, prion diseases were first described as Creutzfeldt-Jacob disease (CJD), a term used to describe a series of degenerative diseases affecting the Central Nervous System. According to Weissmann, et al.\cite{4} also reported cases of Scarpie, documenting it as an infectious disease in 1936. In the 1950s, a new disease known as ‘Kuru’ with similar neuropathology to CJD was discovered in Papua New Guinea. Following increased scientific research, the transmissible ability of both CJD disease and Kuru in Chimpanzees came to be known as transmissible spongiform encephalopathy (TSE) in the 1960s\cite{4}. The discovery of slow virus infections theory by Gajdusek paved way for the discovery of the protein-only hypothesis by Stanley Prusiner credited for the discovery of prions\cite{5}. Prior to Prusner’s proposal, prion diseases were believed to be caused by viruses hence were classified as slow viral diseases. However, Prusiner managed to propose and illustrate a new type of pathogen composed solely of proteins.

**Prion theory**

Prion infectivity occurs through the conformation of prion proteins\cite{6}. Prions are known to be transmissible virus-like agents that lack nucleic acids hence are exclusively composed of modified proteins. The genes that encode prion proteins otherwise known as PRNPs have been identified in both vertebrates and invertebrates, and are present and active in both the brain and other somatic tissues. The set of PRNPs belong to a cluster of differentiation genes known to provide instructions that make proteins on the surface of leukocytes during the developmental stages. Although the precise function of the prion protein is yet to be identified, the National Institute of Health (NHI) theorize that they are most likely associated with key cell functioning mainly cell signaling, cell protection, cell synapse formation and the transportation of copper ions into the cells\cite{7}. Other possible functions of prions may include spatial learning, calcium homeostasis, circadian rhythms and the regulation of anti-oxidative stress.

Prions found in different hosts are unique with each species encoded by a different sequence on the PRNP gene of the animal or human it was last replicated. Illustrating the role of the PRNP gene in prion diseases, Prusiner et al.\cite{5} found that mice that lacked the PRNP gene could not be infected with the misfolded prion protein. Prions also have strain-specific properties which are unique to each prion diseases hence abnormal folding configurations due to the lack of nucleic acid genomes. While the normal prion protein (PrP\textsuperscript{c}) is bound on the surfaces of neurons, infectious prion arises when the normal protein is either misfolded or altered to take a different conformation identified as prion protein scrapie associated (PrP\textsuperscript{sc})\cite{8}.

When the PrP\textsuperscript{sc} enters the brain, it binds to the normal PrP\textsuperscript{c} inducing PrP\textsuperscript{sc} molecules to convert more normal PrP\textsuperscript{c} to the abnormal PrP\textsuperscript{sc} form. According to Biacabe et al.\cite{9}, while it remains unclear on how the conformational change occurs, it is probable that normal PrP\textsuperscript{c}’s take on the abnormal conformation since the abnormal PrP\textsuperscript{sc} activates certain enzymes that modify the normal PrP\textsuperscript{c} structure. It is further probable that the interaction between PrP\textsuperscript{sc} with PrP\textsuperscript{c} results in the cross-linking of the normal PrPc molecules which triggers programmed cell death or apoptosis\cite{8,9}. Although normal, the cross-linked proteins lead to neuron loss while the misfolded or abnormal proteins play the role of infectious agents.

Prusiner et al.\cite{5} was able to prove that the prion was an aberrant, aggregated self-replicating conformer PrP\textsuperscript{sc} of the normal cellular prion protein PrP\textsuperscript{c}. Subsequent researches by Sakudo et al.\cite{10} and Lyketsos and Appleby\cite{11} revealed that, both casual and infectivity misfolding of the \(\alpha\)-helical PrP\textsuperscript{c} entails the enrichment of the PrP\textsuperscript{sc} Isoform whose surface are known to favor the attraction and conformational conversion of PrPc molecules due to their sticky surfaces. The conformational process also leads to elongation with PrPsc functioning as templates that drastically modify the original PrPc biological properties. Research by Katole et. al.\cite{12} further revealed that, the modified PrPc acquires a compacted structure believed to be responsible for prions’ resistance to external harsh environments such as the digestive tracts. The discovery that prions’ oligomers and larger aggregates can fragment prompted Barria et. al.\cite{13} to conclude that prions
have seeding capacity which ultimately deposits outside neurons further affecting new cells. Accumulated PrPsc causes astrocytosis, neuronal cell loss and calculation leading to spongiform and eventually death.

**Molecular biology of prion and prion diseases**

The function of normal prion proteins and the mechanisms by which PrPsc is accumulated thereby leading to neurodegeneration remains the focus of extensive research. Nonetheless the role of host PrPc for prion pathogenesis and propagation has been demonstrated in mice whose PrP genes have been disrupted. Research by Yuan et al.\[14\] found that, mice with disrupted PrP gene were resistant to scrapie infection while when the mourine PrPc transgene was restored, their susceptibility to infection normalized. In humans, the prion protein is mostly expressed in the CNS and the immune system. Prions have a high affinity to copper; besides, it has been found to possess superoxide dismutase activity when refolded in high concentrations of copper chloride. These findings suggest that the PrP gene is possibly a cell-surfaced receptor in addition to being responsible for copper metabolism and/or transport.

Pathogens are disease causing are often transmitted through air, drinking water, food, or body fluids and are of different types such as bacteria, prions, fungi, viruses, protozoa, algae and bacteriophages. Mechanisms of most pathogens are well understood, for example fungi and bacteria form spores under dry or low nutrient conditions, protozoas form cysts while helminthes form eggs\[15\]. Viruses on the other hand inject their genomes into host cells after infection which then matures using the host cell’s machinery. Additionally, different viruses react differently, for example viroids are devoid of proteins while animal viruses can further be divided into enveloped and non-enveloped. Prions, on the other hand, are unique pathogens difficult to classify and inactivate and transmissible through transfusion, eating or neurosurgery\[15\]. The infectivity of prions has raised safety concerns prompting measures such as safe neurosurgery, safe transfusion and food safety.

**Human prion diseases**

The two most common human prion diseases are the Kuru whose mechanism of pathogenesis is ritualistic cannibalism and the Cretzfeldt-Jakob disease (CJD) whose mechanism of pathogenesis remains unknown\[11\]. The sporadic CJD (sCJD), related to CJD also has no known mechanism of infection but believed to be possibly through either the spontaneous conversion of PrPc to PrPsc or possibly through somatic mutation. Other strains of the CJD include familial CJD (fCJD), Variant CJD (vCJD) and Latrogenic CJD (iCJD). vCJD’s infection is believed to occur from consuming BSE...
contaminated cattle products and through secondary blood born transmission. fCJD is occurs due to germline mutations of the PrP gene while iCJD infection occurs from contaminated dural grafts and corneal, infected neurosurgical equipment and pituitary hormone. The two last known prion human diseases both caused by Germline mutations of the PrP gene are the Gerstman-Straussler-Scheinker Syndrome (GSS) and Fatal familial insomnia\(^ {17}\).

Human prion diseases, although not contagious or communicable, should be managed with adequate standard precautions. Based on today’s findings, there exists no evidence of either aerosol or contact transmissions between humans. However, prions are known to be infectious under certain circumstances such as the ritualistic cannibalism practiced in Papua New Guinea known to cause kuru\(^ {1}\). Other known mechanisms of prion transmission in humans include corneal grafts, transplantation of prion-contaminated dura mater and the administration of prion contaminated growth hormone which causes iatrogenic CJD. Research by CJD shows the possibility that variant CJD can be transmitted by blood transmission. However, there exists no evidence that the non-variant forms of CJD can occur due to blood borne transmission. FFI, GSS and Familial CJD are, on the other hand, dominantly inherited prion diseases. According to Donnelly et al.\(^ {17}\) many variant mutations of the PrP gene have been found to be genetically linked to the onset of inherited prion disease.

**Animal prion diseases**

The first animal prion disease to be identified in the early 19\(^ \text{th} \) century was Scapie whose natural hosts are mainly goats, sheep and mouflons. Scapie’s mechanism of pathogenesis is primarily through infections in genetically susceptible sheep. The second animal prion disease is Bovine Spongiform encephalopathy (BSE) which only affects cattle and is transmitted through prion-contaminated feedstuffs. Chronic Wasting disease (CWD) is a fatal neurodegenerative prion disease whose pathogenesis remains unknown and which affects mule, deer, rocky mountain elk and the white-tailed deer. Belay and Schonberger\(^ {1}\) theorizes that CWD is possibly contracted from either direct or indirect contract from both animal contact and contaminated water and feed sources. Exotic ungulate encephalopathy (EUE) is another animal prion disease which mainly affects the Oryx, greater kudu and nyala species. EUE is closely related to Feline spongiform encephalopathy (FSE) which mainly affects the domestic and wild cats in captivity.

Both EUE and FSE are believed to be contracted from BSE contaminated feedstuffs. Another animal prion disease is the Transmissible mink encephalopathy (TME) which mainly affects farm raised minks and is contracted through infection with prion contaminated feedstuffs. The exact mechanism on how prion animal diseases spread among different species remains unknown. However, research by Perrott et al.\(^ {18}\) provided considerable evidence that within sheep and goats, oral inoculation with placental membranes from infected animals was a source of scrapie infections. Other than scrapie, TME, FSE, BSE and EUE are all thought to occur following the consumption of prion infected foods. With regards to CWD, its exact transmission mechanism among Rocky mountain elk, mule deer and the white-tailed deer remains unknown\(^ {11}\).

**Strain typing**

According to Wadsworth et al.\(^ {19}\) PrP produces distinct bands when subjected to the western blot. This is because, prions have sugar molecule at the prion protein glycosylation sites. As a result, treating infected brain homogenates using protease proteinase K within regulated conditions have been found to completely degrade PrPc while leaving PrPsc substantially intact. This is largely an infective treatment option since Proteinase K cleaves amino acids at the N terminus hence has a lower molecular mass. A related research by Salvatore et. al.\(^ {20}\) found that different strains of same prion diseases produced varying migration patterns suggesting different conformations.

The discoveries of different strains and conformations have made it possible to isolate the ratio percentages of proteins in different strains of both variant and sporadic CJD in humans. Wadsworth et al.\(^ {19}\) contends that, in order to encode the diversity of strain type, specific biochemical properties of both the same and different species
should be analyzed. A study by Laplanche et al.[21] on the expression of human PrP in transgenic mice demonstrated that, in the case of CJD, both glycoform ratio and fragment size of PrPsc were maintained. The research further found that transmission of both bovine and human prions to wild-type mice resulted in murine PrPsc with similar fragment sizes and glycoform ratios.

Evidently, it is the PrPsc glycosylation patterns that determine the neuropathological patterns of different prion strains since glycosylation occurs before PrP is converted to PrPsc. Furthermore, different cell types have different mechanisms of glycosylating proteins, for example certain PrPsc glycoforms have been found to replicate most favorably within neuronal populations with distinct PrP glycoform expressed on the cell surface. This specific targeting further accounts for the varied incubation periods evident in different prion strains especially in the regions with higher levels of PrP expression such as the brain regions[19].

**Host genotype**

Evidently, TSEs have the tendency to have both infectious as well as familial etiologies hence understanding the host genotype provides valuable insights on prion diseases. A 1991 study by Palmer et al.[22] on 22 cases of sporadic CJD found that 21 out of 22 of the cases were homozygous for either valine or methionine. Larger subsequent studies by Laplanche et al.[21] and Salvatore et al.[20] both supported the initial results. A study by Collinge et al.[23] which genotyped seven CJD patients undergoing treatment with human pituitary hormones found excess homozygotes at cordon. The findings suggest that, the lack of heterozygosity at cordon 129 is a risk factor for prion diseases. Heterozygosity at cordon 129 possibly reduces prion protein’s ability to refold into structures that propagate CJD prions. Nonetheless, since cordon 129 heterozygotes may still acquire the prion disease, whether this protection is total or merely partial remains unknown.

The existence of ‘species’ barrier that restricts the transmission of different prion strains between species is documented in a number of studies dating back to the 1950s.[21]. Researchers have found that when prions from one species are inoculated into another, the incubation period increases while the number of animals that succumb to the disease decrease. However, when prions are inoculated in animals of the same species, all become sick with very consistent incubation periods. In cases of the second passage when infectious tissues are taken from animals that do not become sick to healthy ones of the same species, the infectivity patterns are similar to transmission within similar species. Inoculating 10-fold serial dilutions to rigorously quantify infectivity revealed that 50% of animals of different species succumbed to the disease[22].

Original explanations for the species barrier hypothesized that, it was due to the differences in the primary structures of PrP in both the originator and host species. Prusiner et al.[5] demonstrated that, when mice which are resistant to hamster prions are modified to express the hamster PrP gene, they become very highly susceptible to hamster prions. A study of both acquired and sporadic CJD transmission of patients homozygous for valine polymorphism at position 129 of the prion gene revealed interesting findings. Palmer et al.[22] found that infections occur most readily when the primary structure of PrPsc is identical to that of the PrPsc responsible for prion propagation. Limited understanding, however, still exist especially with regards to other types of prion diseases. For example, BSE prions have been found to transmit efficiently across different species while maintaining their original transmission characteristics.

Sporadic CJD for example, while readily transmit to certain type of mice, do not easily transmit to wild-type mice. The findings imply that protein conformation is also as important as the primary structure in determining the species barrier. Consequently, a number of researchers have proposed the use of transmission barrier as a replacement of species barrier. Analyzing a number of animals with clinical diseases, Supattapone et al.[24] found that, there existed a number of highly efficient barriers that limited the transmission of prions among mice. Salvatore et al.[20] conclude that, it is possible that the transmission barrier do not just occur as a result of replicating the infectious prions, but also due to subsequent neurodegeneration.
that occurs when the prions are further accumulated.

**Detecting prions**

Based on the currently existing knowledge, the key to therapy for prion diseases lies in early diagnosis before extensive neurological impairment has occurred. Over the past three decades, major increases in the understanding of both pathology and etiology of prion diseases have improved management options. Diagnosis was originally made by looking for spongiform changes and amyloid plaque depositions on histology. Hence, the presence of infectious prions was only determinable by demonstrating the passage of brain homogenate to indicator animals, a tedious, lengthy and costly process. Although the method is still in use, the fact that infectious prions produce PrP specific antibodies has allowed for the addition of immunohistochemistry to the histologist’s arsenal. This has served to reduce cost and time of diagnosis while improving diagnosis specificity.

Animal prions are often detected by culturing a homogenate prepared by treating infected brain tissues with Prteinase PK. The culture is then applied to microtiter plate for absorption before using anti-PrP antibody to detect prions. To validate results, enzyme linked immunosorbent essay (ELISA) is repeatedly performed. Additional detection options include immunohistochemistry and western blotting. Western blotting uses a specialized membrane to absorb PK-treated proteins that have been separated by sodium dodecyl sulfate (SDS) through the polycrylamide gel electrophoresis (PAGE) process. Following the absorption of PK-resistant PrP, specific membrane-bound proteins are then detected using specialized anti-PrP antibody. The western blotting diagnostic technique also provides information on mobility of peptides, which is often influenced by prion strains and the host genotype.

The immunohistochemistry (IHT) is often carried out based on representative pathological features of specific prion diseases inclusive of neural cell loss, astrocytosis, amyloid plaques and vaculation. IHC analysis of specific brain sections, neural cell loss, determining the accumulation of PrP amyloid plaques and astrocytosis are carried out using light microscopy. Peggion et al.\(^\text{[15]}\) contends that, despite vaculation being used as an index of prion infections, certain combinations of different prion strains with host species have been found to lead to accumulation of PrP without vaculation in brain sections. Another more recent detection technique is the protein misfolding cyclic amplification method (PMCA) developed by Saa et al.\(^\text{[6]}\) PMCA enables *in vitro* amplification of small quantities of PK-resistant PrP as the seed through sequential cycles of incubation and sonication.

Supporting research by Katole et. al.\(^\text{[12]}\) further found that, the levels of PrPres amplified by the PMCA method directly correlated with prions infectivity titer besides the method has been found to detect prions in the blood. Additionally, PMCA method can be used to diagnose both pre-symptomatic and terminally diseased hamsters. Comparing all known prion detection methods, PMCA currently has the highest sensitivity for detecting PrP\textsuperscript{res}. The method is extensively used in detecting prion related diseases in goat, cattle and sheep and is effective in sCJD, hamster scrapie, CWD, and vCJD. Modified PMCA versions have been designed by combining PrP-PMCA and using quaking-induced conversion (QUIC) reaction.

**Antiprion therapeutics**

Katole et. al.\(^\text{[12]}\) documents a number of compounds known to interact with PrPsc. These include dextran sulphate, anthracycline, congo red, polyanions, \(\beta\)-sheet breaker peptides and pentosan polysulphate. The compounds, are however, only effective if administered before the onset of clinical diseases. The compounds have also been noted to exhibit either very high levels of toxicity or low levels of bioavailability. Researchers agree that an effective antiprion therapy should be able to bind PrPsc thereby preventing further conversion of PrPc hence managing the disease’s progression. Peggion et. al.\(^\text{[15]}\), however, disagrees noting that this approach is unlikely to lead to a cure hence there is need for a clearer understanding of PrPc as well as methods that allows early diagnosis.

Presently, clinical trials using chlorpromazine and quinacrine for the treatment of CJD and vCJD are underway in the UK and US. Although the results are still unknown, there is evidence that the drugs
could be useful against prion disease in vivo. Researchers, however, find it challenging to transfer in vitro experiments to a clinical setting. Both Yuan et al.\cite{14} and Sakudo et al.\cite{10} agree that, there is increased public demand for any treatment able to either slow down or completely eradicate these extremely distressing and fatal diseases. In 2002 for example, there was a legal challenge that obliged a UK health authority to administer intraventricular pentosan polysulphate, an experimental drug, to two health patients. Researchers

Prion sterilization

Prions are extremely difficult and resistant pathogens that are not effectively inactivated using conventional sterilization procedures. Thorne et al.\cite{25} documents four cases in which iCJD was caused by neurosurgery in the 1980s hence the need for specialized treatment of surgical equipment especially those used for spinal surgery, craniotomy and ophthalmic surgery. The difficulty in sterilizing prions is due to the fact that, these pathogens have no nucleic acid hence cannot be inactivated by methods such as exposure to UV, γ-ray radiation, alcohol treatment, and autoclaving (121°C, 20 min)\cite{7}. Activating prions need autoclaving under severe conditions for example autoclaving under (134°C, 18 min), NaOH (1 N, 20°C, 1 h), SDS (30%, 100°C, 10 min), and NaOCl (20000 ppm, 20°C, 1 min) is especially recommended. The appropriate procedure recommended by Sakud and Shintani\cite{7} begins with washing with appropriate detergents followed by SDS treatment 3% for 3-5 minutes. This is followed by treatment with alkaline detergents (80-93°C, 3-10 min) followed by autoclaving (134°C, 8-10 min). The apparatus is then washed in alkaline detergents at recommended concentrations before being vaporized using hydrogen peroxide gas plasma sterilization.

CONCLUSION

The abnormal PrPsc are known to cause a range of prion diseases such as scrapie, bovine spongiform encephalopathy (BSE) and Creuzfeldt-Jacob disease (CJD). PrPsc is derived from PrP normally expressed by the host cells and whose normal function is relatively unknown. Prions have the unique feature of lacking nucleic acid making its inactivation challenging and requiring different procedures from those used in other pathogens. Besides, prion diseases are zoonotic infectious diseases for example BSE prion which can be transmitted via blood is known to cause vCJD in humans. Although recent research has led to drastic improvements enabling early and more accurate diagnosis, majority of existing diagnostic methods of a range of prion diseases remains post-mortem. Analyzing the different diagnostic options, PMCA raises the possibility of pre-mortem diagnosis for both preclinical onset and terminally ill patients with the use of the blood samples. Finally, since prions are known to be notoriously resistant pathogens difficult to inactivate with conventional sterilization procedures such as alcohol treatment and autoclave, more advanced methods such as autoclaving under severe conditions and gas plasma sterilization should be used.

CONFLICT OF INTEREST

None.

REFERENCES


