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An Overview of Prion Diseases: Protein Transformation, Effects on Animals, and Current Treatments

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ABSTRACT

Prion disease is an extremely rare disease that occurs in about 1 in a million people, and people usually die in a couple of months to years following diagnosis. This disease predominantly affects the population older than 60, as the symptoms appear more frequently at this age. The distribution of this disease is completely random, and there is no specific area of distribution of this disease in the world except kuru. Kuru is in Papua New Guinea. However, the emergence of the prion disease occurs well before in the nervous system. There are cases of prion disease occurring in other parts of the body, but it primarily develops in the nervous system. One single abnormal prion protein can transform the entire environment of the human body, and terrifyingly, it can be spontaneous. Although the disease is rare it is unpredictable, and anyone can have it. Especially with the abnormal prion protein's resistance to "death", there is no stopping once the first prion protein has transformed and seeded in the body. Without the complete knowledge of the prion disease and the mechanism that causes it in the first place, there is no absolute cure for this disease. The knowledge related to even knowing the complete mechanism and duty of the normal prion protein is unknown. What has been studied is that the gene responsible for the prion protein synthesis has been highly conserved in mammals, indicating an evolutionary importance. Nonetheless, other mammals are in danger of prion disease. As more mutants of prion disease grow in animals, humans are more in danger of contracting it, such as the outbreak in England in 1986. Although this outbreak was not a large outbreak, people unfortunately fell victim to the disease. Furthermore, this predicts future epidemiological issues that can occur without a clear cure for prion disease.

Introduction

Caused by the overtime accumulation of the abnormal prion protein; prion diseases, also referred to as transmissible spongiform encephalopathies (TSEs), are infrequent but lethal brain disorders that impact humans and a wide variety of animals. These disorders are caused by the transformation of the PrP^{C} prion protein to a PrP^{Sc} prion protein. The normal prion protein is responsible for a range of functions in the brain and regulates neuronal homeostasis, but the complete function of the enigmatic protein is yet to be discovered. These disorders are also prevalent in animals, such as cows, sheep, and most currently, camels. Currently, full-recovery treatments are non-existent, and only short-term treatments that alleviate the pain exist. In majority cases, prion disease has a 100% mortality rate, but the range of life expectancy in some other cases depends on the type of prion disease (Geschwind, 2016, p. 1). Prion diseases are classified into three major types, which are sporadic, genetic and acquired. The most common type is sporadic prion diseases in humans, an example being the Creutzfeldt-Jakob disease. The prion disease classification differs in animals, as most animals are infected with the acquired classification of prion diseases. In some rare cases, prion diseases

can also be transferred from animals to humans, especially from cattle that are affected by diseases like mad cow's disease. This research paper is focused on providing an overview of prion disease based on current knowledge and their impact on mankind and therapeutic options.

What are prion diseases and how do they form?

Prion diseases are neurodegenerative diseases that occur because of the transformation of the prion protein. The prion protein, denoted as PrP^{C}, which stands for "prion related protein" with the "C" standing for "cellular form of the protein"(Geschwind, 2016,p. 2) , is structured as a disordered N-terminal domain and a globular C-terminal domain composed of three alpha-helices and a short beta-rich sheets. The alteration process of the PrP^{C} is known as the "prion hypothesis". The prion hypothesis claims that the infectious agent for prion disease is a protein that does not contain any nucleic acids (Sigurdson, 2019). This is supported by an experiment done by the British scientist Tikvah Alper, who used ionizing radiation to find the measurements of the infectious agent in the scrapie disease, a prion disease found in sheep and goats. The radiation used in the experiments would kill nucleic acids, however, Alper found that this was not the case as the activity of the infectious agent was still continuing. The protein that causes prion disease is the misfolded PrP^{Sc}(Geschwind, 2016). The PrP^{C} changes structure from a mostly alpha-helical protein into a PrP^{Sc} , which is a highly beta-sheet-rich structure. The PrP^{Sc} is also partially protease-resistant, as opposed to PrP^{C}. Since it is resistant to protease, the PrP^{Sc} is immune to the protease enzyme that degrades proteins, which explains its highly contagious feature (Sigurdson, 2019).

However, the concealed mechanism is still not known till date. One hypothesis, according to a mathematical model created by Griffth, to actually turn into PrP^{Sc}, the PrP^{C} must go through an intermediate step. The intermediate step is the PrP state, a reactive state where the structure of the PrP^{C} is relatively unfolded. To even reach a point where the PrP^{C} is in the PrP state, exogenous energy is needed to overcome the large energy barrier between PrP^{C} and PrP^{Sc}, as seen in Figure 1. Sometimes, the PrP-binding molecules lower the activation energy needed to overcome the barrier, essentially allowing the conversion of the PrP^{C} to PrP^{Sc} to occur (Ma,2014). Figure 1 indicates the process of conversion of PrP^{sc} to PrP^{c} and the amount of energy needed for this process.

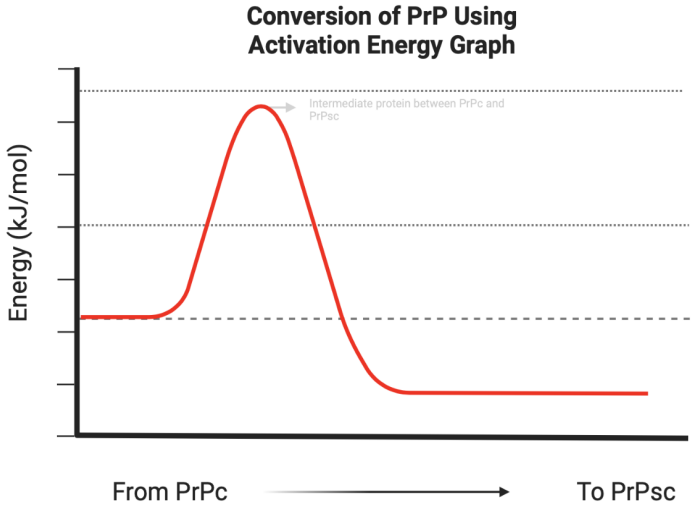

Figure 1. Transformation of the PrP^{sc} to PrP^{c} using energy. In this transformation process, the prion protein must overcome an energy barrier, however, this is near impossible because of the enormous amount of energy needed. After it overcomes the barrier, the prion protein stays in an intermediate stage, before turning into the abnormal PrP^{Sc}. This graph shows the amount of energy needed to become a PrP^{Sc}. However, this is also a theory, and this idea may not be plausible.

To add on to the hypothesis, another hypothesis involves the process of the PrP^{Sc} actually converting the PrP^{C}. Essentially, this hypothesis is the aftermath of the "first" conversion. In this hypothesis, the beta-sheets of the PrP^{Sc} interact with the PrP^{C}, forming a complementary amino acid side chain. Through hydrogen bonding, the two beta-sheets lock together and stabilize the growing fibrils, which are the elongated protein structures (Sigurdson, 2019).

Structure of prion proteins

The key difference between PrP^{C} and PrP^{Sc} is the substantial structural change that goes from the mostly alpha-helical protein to a highly beta-sheet-rich protein as seen in Figure 2. The transformation is most likely converted in MVB's (multivesicular bodies), as the stopping MVB maturation reduces PrP^{Sc} production (Sigurdson, 2019). Mammals share these common features in the two proteins, with some differences. The PrP^{C} is a globular structure, which can be described as a "sphere" and includes different levels within. The N-terminal, which is the start of a protein, has two definite and preserved regions that are present through all animals with the PrP^{C}. One of the two regions contains a "segment of five repeats of eight amino acids sequence" (Tian, 2013). This segment of the PrP^{C} allows for bonding with divalent metal ions. The process of binding to divalent metal ions could have consequences that may develop the prion disease, however, that has not been clearly established. The other region has been regarded as the conserved region PrP^{C}, and is highly water-resistant or hydrophobic. This region also acts as the transmembrane region of PrP^{C}. In comparison to the PrP^{Sc}, the protein as mentioned before, is extremely protease resistant as mentioned before. Additionally, the PrP^{Sc} is also insoluble, meaning it cannot dissolve in liquids. The PrP^{Sc} also rearranges the disulfide bonds that are initially found in the PrP^{C} (Tian, 2013).

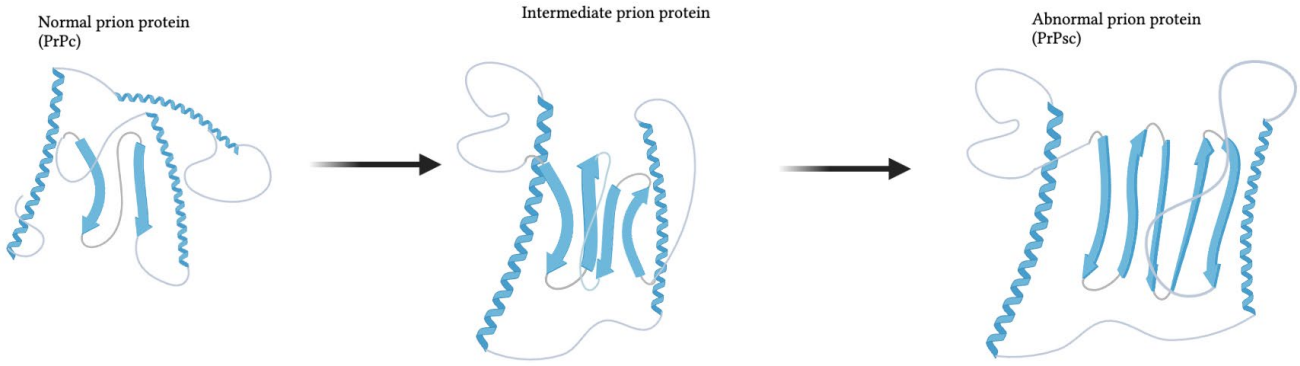

Figure 2. Transformation of the prion protein. The picture above shows the transformation of the prion protein (PrP^{c}) to the abnormal prion protein (PrP^{sc}). The process includes three steps. The first step includes the normal prion protein, which is structured as mostly alpha helix with beta sheets. The next step is the intermediate step. This structure is overrun by the beta sheets, and the alpha helix percentage decreased significantly. The last step includes the final product, which is the PrP^{Sc}. The PrP^{Sc} primarily contains beta sheets, with alpha helix. This represents the protein misfold that occurs within prion disease.

Types of Prion diseases

Prion diseases are categorized into three distinct subtypes. These subtypes describe how the normal PrP^{C} changes to the abnormal PrP^{Sc}, and are related to the prion protein gene (PRNP), which is responsible for the prion protein. The subtypes of prion disease are dependent on how the PRNP gene is changed, which in turn changes the PrP^{C}. PRNP has been conserved for centuries in mammals, implying the noteworthiness of the prion protein itself in the human body. In all mammals and avians, the PRNP is located in a single exon. The exon is part of a DNA that contains instructions to translate into a protein or peptide chain. In humans, "an untranslated 5′ exon" (Tian, 2013) was also present in genomic DNA. A 5'exon is the leader sequence. This discovery was also found in hamsters PRNP as well, giving validation to the fact that the PRNP is conserved in animals. The PRNP contains many guanine-cytosine (GC) repeats. This site binds to the Sp1 transcription factor that regulates the transcription of genes. Because of this, the PRNP expresses in various different sites, such as "brains, muscles, and some immunocytes," (Tian, 2013). However, the highest amount is found in neurons and highly associated with the prion disease (Tian, 2013).

Sporadic

Sporadic subtypes are the most common out of all three types of prion diseases in humans. About 85-90% of all diagnosed cases of prion diseases are sporadic. Sporadic prion disease is the spontaneous transformation of the PrP^{C} to a PrP^{Sc}, and there are no external factors that were identified to cause this transformation. The unpremeditated conversion of the PrP^{C} causes the inflation of PrP^{Sc} overtime and leads to a clinical diagnosis of prion disease. One of the most common types of sporadic prion disease is the sporadic Creutzfeldt–Jakob disease (sCJD). Most patients die within 12 months of the diagnosis, and symptoms include cognitive impairment. A genetic marker of sCJD is the variation or polymorphism at codon 129 of the PRNP. This changes the type of protein being made. The symptoms in this type of prion disease are similar to that of schizophrenia or dementia and muscle atrophy. Another type of sporadic prion disease is sporadic Fatal Insomnia (sFI) and occurs similarly to sCJD. The key difference is the disease symptoms. In sFI, the patient typically experiences the difficulty falling asleep for months on end, until they lose their consciousness and unfortunately die (Will, 2017).

Genetic

In humans, the genetic subtype is the second most common out of all three prion disease types. Genetic prion diseases are caused by mutations in PRNP. The mutations can either be "missense, nonsense, and/or octapeptide repeat insertions or, possibly, deletions" (Kim, 2018). For diseases such as familial Creutzfeldt–Jakob disease (fCJD) and Gerstmann –Sträussler–Scheinker disease (GSS), the most common type of mutation is the octapeptide repeat insertions (OPRI). Genetic types are classified by their progression in a person, which can have variable progression patterns. One manifestation of the genetic prion disease can be the familial Creutzfeldt–Jakob disease, which is very similar to the sporadic counterpart. In familial Creutzfeldt–Jakob disease, the patient exhibits dementia-like symptoms, and usually lives about a year after the diagnosis. People with genetic prion disease are diagnosed with mutations in the PRNP gene. In case of fCJD disease, "D178N codon 129V PRNP mutation" was observed in PRNP gene (Kim, 2018), and is an hereditary disease. Gerstmann –Straüssler–Scheinker disease (GSS) is another type of genetic prion disease which involves poor muscle control, and is another hereditary disease caused due to the P102L mutation in PRNP gene. The final type of genetic prion disease is fatal familial insomnia. In this, the thalamus, a part of the brain that plays a huge role in the process of sleep, is essentially deteriorated, and is caused due to the D178N mutation in PRNP gene (Kim, 2018).

Acquired

Acquired prion disease is the least common in all the prion disease types, accounting for less than 1% of all prion disease cases in humans. Acquired prion disease is usually attained by the transfer of some type of brain matter that was already contaminated with prion disease. One example of acquired prion disease was Kuru, occurring within the small Fore tribe in Papua New Guinea. Demographically the disease was most common in women and children and was most likely obtained by the cannibalism ritual performed by the tribe, where women and children ate the brain tissue. Presumably, one of the brains that were consumed by the women and children was already infected with a prion disease such as sCJD, which caused the kuru outbreak (Will, 2017).

Prion Diseases in Animals

Similar to prion diseases observed in humans, several diseases were also observed in mammals. Prion diseases are also triggered by the conversion of the PrP^{C} to the PrP^{Sc} -in animals, and the drastic changes are observed in the shape of the protein. Scientists found the changes to the PrP in samples collected from animals with scrapie disease. Scrapie primarily impacts goats, sheeps, and other small ruminants. The prion hypothesis that described that conversion of PrP^{c} to PrP^{sc} is the primary cause of the prion disease was observed from the scrapie disease and provided conclusive evidence. It was the first prion disease discovered by humans in animals. Symptoms of this disease among mammals include tremor, temperament changes, and extreme muscular atrophy. Another prion disease, bovine spongiform encephalopathy (BSE), found in cattle, became important, not only because of the value of cattle in the economy and zoonotic nature of the disease to jump from animals to humans. BSE was transferable to humans and caused a small outbreak in England. The symptoms of BSE disease are similar to scrapie disease in that it causes temperament changes, abnormal posture, and muscle atrophy. In humans, the symptoms of prion diseases are quite similar to the ones observed in animals. In addition, other symptoms observed in humans such as intellectual and memory loss, muscle atrophy, and increasing loss of brain function were also observed in prion diseases in animals (Gough, 2015).

Reasons that contribute to prion diseases in animals

Again, similar to humans, the change in the PrP^{C} to PrP^{Sc} is the main catalyst for prion diseases in animals. The structure of the prion protein is essentially the same in all mammal species, which is mostly alpha-helical (40%) with beta-sheets (3%). With the conversion, the PrP^{Sc} is now structured as mostly beta-sheets (45%) and less alpha-helical (35%). Like humans, the PrP^{Sc} is partially resistant to protease digestion, which leads to accumulation of abnormal prion protein. The vulnerability of prion diseases in animals is also related to the polymorphism of the PRNP gene, but it differs from codon 129 in humans but can be contributed by polymorphisms in codons 136, 154, and 171 of scrapie diseased animals (Gallardo, 2021) . This vulnerability is mostly seen in scrapie diseased animals, but other prion diseases in animals aren't connected to a specific gene polymorphism. The diffusion of the prion disease is generally by the "oral route" or also called as a contamination route of transmission (Houston, 2019). BSE, mostly found in cattle, was transmitted through the food the cattle were fed, which contained meat and bones. In some cases, BSE among cattle was transmitted from mother to offspring, or vertical transmission (Gallardo, 2021).

Relation between prion diseases in humans and animals

There have been no recorded instances of the transfer of prion disease between human to animal or vice versa, until 1996. The classical bovine spongiform encephalopathy (C-BSE) has been stated to be the provoker of the new variant Creutzfeldt-Jakob disease (vCJD). Unfortunately, this led to a health crisis, especially throughout Europe, where they

were able to identify the degree of prion disease situations in cattle, as well as implement action to stop the further spread. When cats were discovered to have prion disease, including research that supports the idea that C-BSE is a disease that can be transmitted to other primates such as the marmoset. In 1995, these concerns were proven when two teenagers from the United Kingdom contracted a new type of prion disease, the vCJD. Further research using mice proved that the symptoms displayed by mice with vCJD were the same as symptoms of BSE and feline spongiform encephalopathy (FSE). Further analysis of infected humans of vCJD indicated that the PRNP gene they had was "homozygous for methionine at codon 129" (Houston, 2019). Other animal prion diseases may have zoonotic potential, such as the atypical scrapie. Atypical scrapie differs from normal scrapie because the ARR allele, which controls the resistance to classical scrapie, does not affect nor control atypical scrapie. Atypical scrapie can either be sporadic or acquired. Although atypical scrapie has never been experimentally transferred to primates, it was transferred to mice. Although not as certain as C-BSE, it is plausible that atypical scrapie can be transferred to humans (Houston, 2019).

Therapeutic options to treat Prion diseases

Since the main site for prion diseases is the brain/central nervous system, most treatments need to cross the "blood–brain barrier (BBB) (Giles, 2017). One type of therapy used in mice was the use of polyanions. Large polyanionic compounds included "sulfated glycans dextran sulfate 500 (DS500) and pentosan polysulfate (PPS) (Ehlers and Diringer 1984)" (Giles, 2017) . The use of this treatment improved the survival time of the patients. Another type of therapy used was small-molecular therapies. One product was the Congo red, an organic compound. The Congo red was able to increase survival minimally in mice, however, this only worked because the mice were injected with Congo red relatively quickly after being injected with prions (Giles, 2017). Other limitations with this form of treatment is that difficulty in translating this therapy from mice to humans, and the prion protein structure varies from human to mice. Another form of treatment that could be used is reducing and eliminating the prion protein altogether. One study conducted on cell cultures includes the "anti-PrP monoclonal antibodies" (Krance 2020). Monoclonal antibodies are synthetic molecules that serve as an antibody that binds to cognate antigen and inactivate its activity. In this case, the monoclonal antibody would target the PrP^{C} for inactivation. This type of therapy is still ongoing with a patient, as this form of treatment is incredibly risky, and does not have any effect after a patient is diagnosed with the prion disease. Several other prion disease specific therapeutic molecules are still currently under clinical trials. Likewise, nucleic acid-targeting therapies (Krance 2020) are used as another form of treatment utilizing shRNA used to silence PRNP genes. The end results of this type of treatment indicate that it prevents the further spread of the PrP^{Sc}, and does not completely stop it following disease diagnosis. Essentially, it is like the monoclonal antibody method, but it targets the gene instead of the prion proteins itself. This type of therapy could be more effective in stopping the production of the proteins, but it still works best for the genetic type of prion diseases. These treatments are breakthroughs that get scientists closer to finding a definitive cure for prion disease, however, the limitations greatly outweigh the pros in these therapies (Krance 2020).

Using cerebrospinal fluid biomarkers to diagnose prion disease

Cerebrospinal fluid (CSF) is that flows in the hollow spaces in between the brain and the spine. CSF biomarkers are used to detect changes in the substances or proteins made by neurons. The important strategy to diagnose prion disease is through the use of CSF biomarkers to detect any changes in the prion proteins being formed and released into the brain microenvironment. The real-time quaking-induced conversion (RT-QuIC) method uses CSF to diagnose sporadic CJD. According to Figure 3, the first step indicates the extraction of cerebrospinal fluid through the process of a lumbar puncture in the spine. The lumbar puncture extracts prion proteins (step 2) that could be either PrP^{C} or PrP^{Sc},

and adds them in a mixture (step 3). The mixture contains recombinant prion protein (rPrP) and thioflavin T. Thioflavin T is a fluorescent dye that is useful in that it expresses the binding of proteins. In step 4, the thioflavin T fluorescence, and this is because the rPrP and the PrP^{Sc} start to form agglomerates. If the prion proteins in the initial sample are PrP^{C}, there would not be any glow. This process takes up to over 30 hours, and most of the time is being spent on the agglomerates that form slowly as the PrP^{Sc} binds to rPrP (Green, 2019). With tests conducted by Ryuichiro Atarashi, this method has been proven to have more than an 80% accuracy rate with diagnosis (Atarashi, 2011).

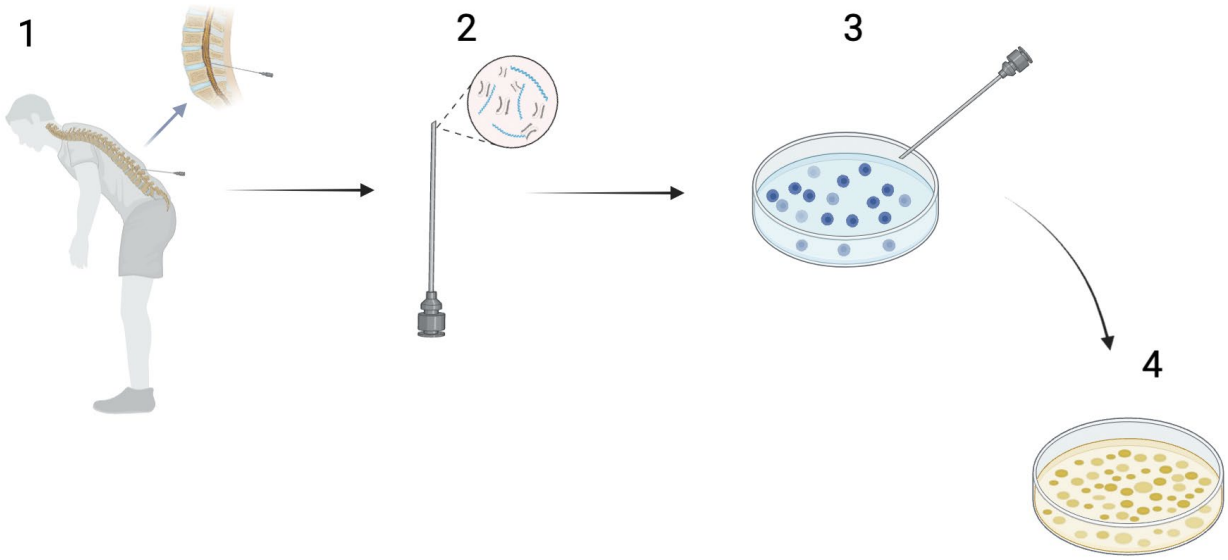

Figure 3. RT-QuIC diagnosis. This is currently the method of diagnosis in presumed sporadic subtypes of prion disease. It is most used for sCJD. This method extracts cerebrospinal fluid, which contains prion proteins. These proteins can either be PrP^{C} or PrP^{Sc}. The extraction is added to a mixture containing PrP and thioflavin T. After an incubation period, which is more or less 30 hours, it can be diagnosed if a patient has prion disease depending on the thioflavin T fluorescence.

Results

To date, there are no current therapies that completely cure prion diseases, and all forms of prion diseases inevitably cause death. The best interests of professionals dealing with patients who are diagnosed is to reduce the amount of pain they are experiencing as a major symptom. Although there are many theoretical treatments that can be further explored as therapeutic option, for example, reducing the amount of prion protein., This can be a risky trial because scientists do not know the actual function of the prion protein and depleting the protein may have effects to impair normal functions of the prion proteins. However, studies that improved our understanding on prion protein showed that the PrP^{C} may not have normal functions. The process of eliminating PrP^{C} must be performed before the diagnosis of prion disease, as performing this procedure after disease diagnosis will only halt the spread of the disease and not terminate it completely. Essentially, this treatment is best for genetic prion diseases, which occurs far less frequently than the sporadic type of prion disease, which is credited up to 80-85% of all diagnosed prion diseases. Since there are three different types of prion diseases, a single approach cannot work efficiently to control all types of prion diseases and type specific approaches may be explored to treat them. The types of prion diseases also pose a greater challenge, as treatments for the sporadic and acquired subtypes are yet to be researched in detail (Krance, 2020).

Discussion

The best way to cure prion disease is to eradicate prion protein completely, as the role of the protein is believed to be futile. This form of treatment can occur with monoclonal antibodies. However, the side effects of this type of treatment are unknown. Even though prion disease is one of the rarest diseases and most people are not likely to be diagnosed with the disease, it would be best to take a course of action that eliminates prion proteins. Other research papers on prion proteins conclude there are other ways to treat prion disease in a patient.

Conclusion

Prion disease is a brain disorder classified into three types, and it occurs not only in humans but also in mammals due to the conversion of PRNP gene transformation from PrP^{C} primarily alpha-helical structure to a PrP^{Sc} majority beta-sheet structure. There are three different types of prion diseases, and each one of them requires a separate type of treatment as they are caused by different sources. Prion diseases can theoretically be transmitted between humans and animals, which has been proved with classical BSE and may occur with atypical scrapie prion disease. Currently, methods for a full recovery don't exist, and current therapies only alleviate the pain. Prion disease is diagnosed with CSF biomarkers in the human brain. The treatments that are being investigated to treat genetic prion disease fail to treat sporadic and acquired types of prion diseases. However, acquired prion disease is ubiquitously observed among mammals and humans. In summary, though some treatment options are available to treat genetic prion diseases, treatments that are effective to treat sporadic and acquired prion diseases need to be still developed by scientists. Novel treatments are essential, especially with future zoonotic nature of the disease and epidemiological concerns, and to improve health of patients suffering from fatal prion diseases.

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