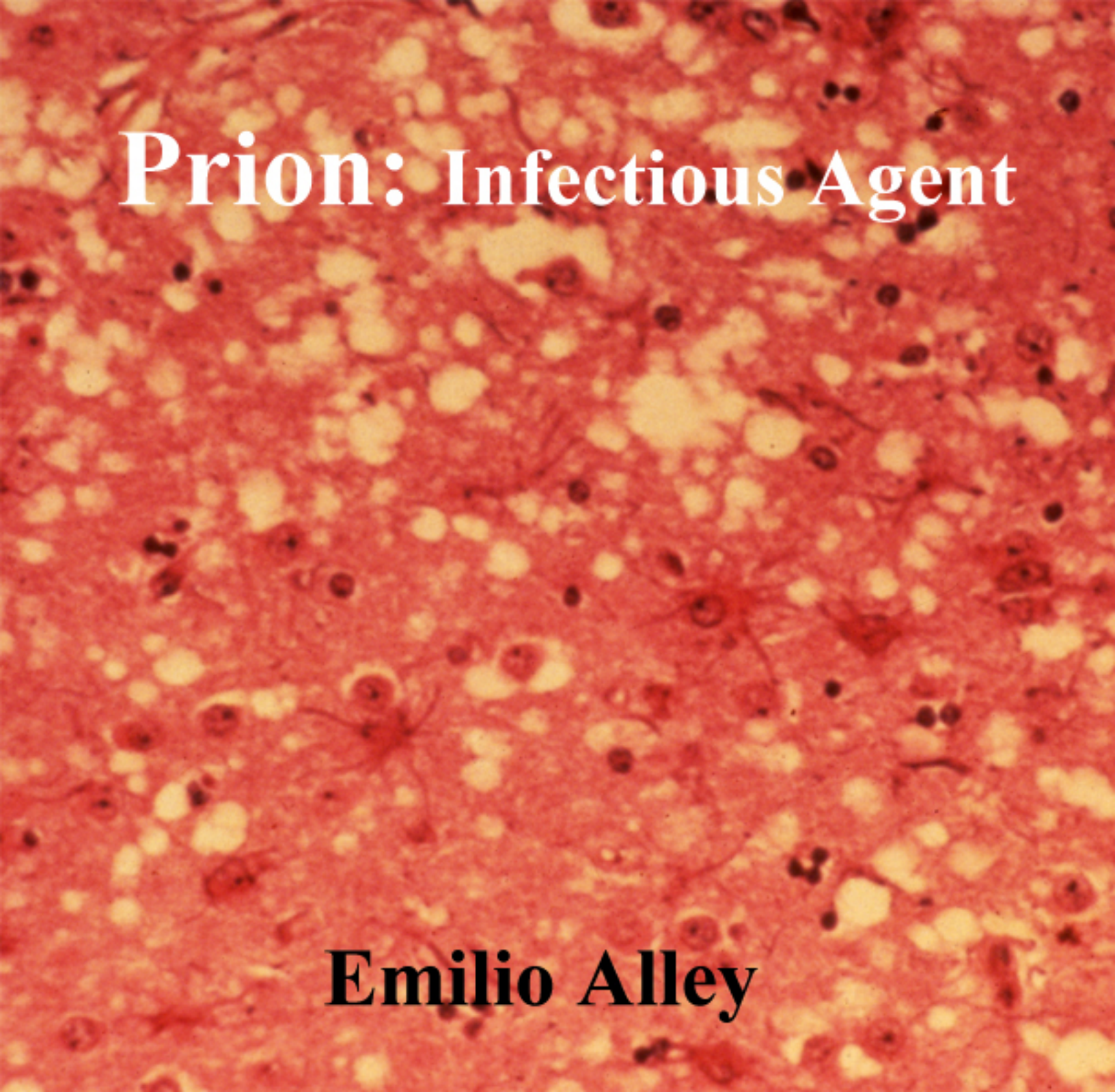

# Prion: Infectious Agent

Emilio Alley

First Edition, 2012

ISBN 978-81-323-3205-3



*Published by:*
**Research World**
4735/22 Prakashdeep Bldg,
Ansari Road, Darya Ganj,
Delhi - 110002
Email: info@wtbooks.com

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# Prion

| Prion Diseases (TSEs) |
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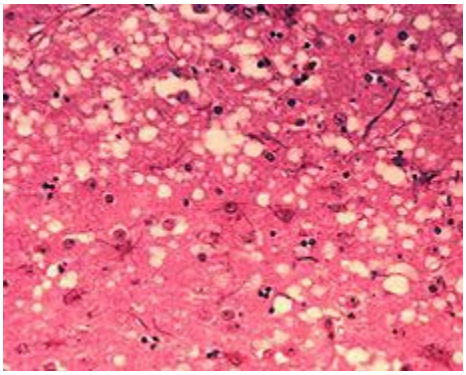


Microscopic "holes" are characteristic in prion-affected tissue sections, causing the tissue to develop a "spongy" architecture.

**ICD-10** A81

**ICD-9** 046

A **prion** is an infectious agent composed of protein in a misfolded form. This is in contrast to all other known infectious agents, which must contain nucleic acids (either DNA, RNA, or both) along with protein components. The word **prion,** coined in 1982 by Stanley B. Prusiner, is a portmanteau derived from the words *protein* and *infection*. Prions are responsible for the transmissible spongiform encephalopathies in a variety of mammals, including bovine spongiform encephalopathy (BSE, also known as "mad cow disease") in cattle and Creutzfeldt–Jakob disease (CJD) in humans. All known prion diseases affect the structure of the brain or other neural tissue and all are currently untreatable and universally fatal.

Prions propagate by transmitting a misfolded protein state. When a prion enters a healthy organism, the prion form of a protein induces existing, properly-folded protein to convert into the disease-associated, prion form; the prion acts as a template to guide the

misfolding of more protein into prion form. These newly-formed prions can then go on to convert more proteins themselves, this triggers a chain reaction that produces large amounts of the prion form. All known prions induce the formation of an amyloid fold, in which the protein polymerises into an aggregate consisting of tightly packed beta sheets. Amyloid aggregates are fibrils, growing at their ends, and replicating when breakage causes two growing ends to become four growing ends. The incubation period of prion diseases is determined by the exponential growth rate associated with prion replication, which is a balance between the linear growth and the breakage of aggregates. (Note that the propagation of the prion depends on the presence of normally-folded protein in which the prion can induce misfolding; animals which do not express the normal form of the prion protein cannot develop or transmit the disease.)

This altered structure is extremely stable and accumulates in infected tissue, causing tissue damage and cell death. This structural stability means that prions are resistant to denaturation by chemical and physical agents, making disposal and containment of these particles difficult. Prions come in different strains, each with a slightly different structure, and most of the time, strains breed true. Prion replication is nevertheless subject to occasional epimutation and then natural selection just like other forms of replication. However, the number of possible distinct prion strains is likely far smaller than the number of possible DNA sequences, so evolution takes place within a limited space.

All known mammalian prion diseases are caused by the so-called prion protein, PrP. The endogenous, properly-folded, form is denoted $PrP^{C}$ (for ***c****ommon* or ***c****ellular*) while the disease-linked, misfolded form is denoted $PrP^{Sc}$ (for ***Sc****rapie*, after one of the diseases first linked to prions.) and neurodegeneration. The precise structure of the prion is not known, though they can be formed by combining $PrP^{C}$, polyadenylic acid, and lipids in a Protein Misfolding Cyclic Amplification (PMCA) reaction.

Proteins showing prion-type behavior are also found in some fungi, which has been useful in helping to understand mammalian prions. Interestingly, fungal prions do not appear to cause disease in their hosts and may even confer an evolutionary advantage through a form of protein-based inheritance.

## *Discovery*

Radiation biologist Tikvah Alper and mathematician John Stanley Griffith developed the hypothesis during the 1960s that some transmissible spongiform encephalopathies are caused by an infectious agent consisting solely of proteins. Their theory was developed to explain the discovery that the mysterious infectious agent causing the diseases scrapie and Creutzfeldt–Jakob disease resisted ionizing radiation. A single ionizing "hit" normally destroys an entire infectious particle, and the dose needed to hit half the particles depends on the size of the particles. The data suggested that the infectious agent was too small to be a virus.

Francis Crick recognized the potential importance of the Griffith protein-only hypothesis for scrapie propagation in the second edition of his "Central dogma of molecular

biology": while asserting that the flow of sequence information from protein to protein, or from protein to RNA and DNA was "precluded". He noted that Griffith's hypothesis was a potential contradiction (although it was not so promoted by Griffith). The revised hypothesis was later formulated, in part, to accommodate discovery of reverse transcription by Howard Temin and David Baltimore.

Stanley B. Prusiner of the University of California, San Francisco announced in 1982 that his team had purified the hypothetical infectious prion, and that the infectious agent consisted mainly of a specific protein – though they did not manage to isolate the protein until two years after Prusiner's announcement. Prusiner coined the word "prion" as a name for the infectious agent. While the infectious agent was named a prion, the specific protein that the prion was composed of is also known as the **Pr**ion **P**rotein (PrP), though this protein may occur both in infectious and non-infectious forms. Prusiner was awarded the Nobel Prize in Physiology or Medicine in 1997 for his research into prions.

Reported January 2011, researchers discover prions spreading through airborne transmission on aerosol particles, in an animal testing experiment focusing on scrapie infection in laboratory mice.

## *Structure*

### Isoforms

The protein that prions are made of (PrP) is found throughout the body, even in healthy people and animals. However, PrP found in infectious material has a different structure and is resistant to proteases, the enzymes in the body that can normally break down proteins. The normal form of the protein is called **$PrP^{C}$**, while the infectious form is called **$PrP^{Sc}$** — the *C* refers to 'cellular' or 'common' PrP, while the *Sc* refers to 'scrapie', a prion disease occurring in sheep. While **$PrP^{C}$** is structurally well-defined, **$PrP^{Sc}$** is certainly polydisperse and defined at a relatively poor level. PrP can be induced to fold into other more-or-less well-defined isoforms in vitro, and their relationship to the form(s) that are pathogenic in vivo is not yet clear.

### $PrP^{C}$

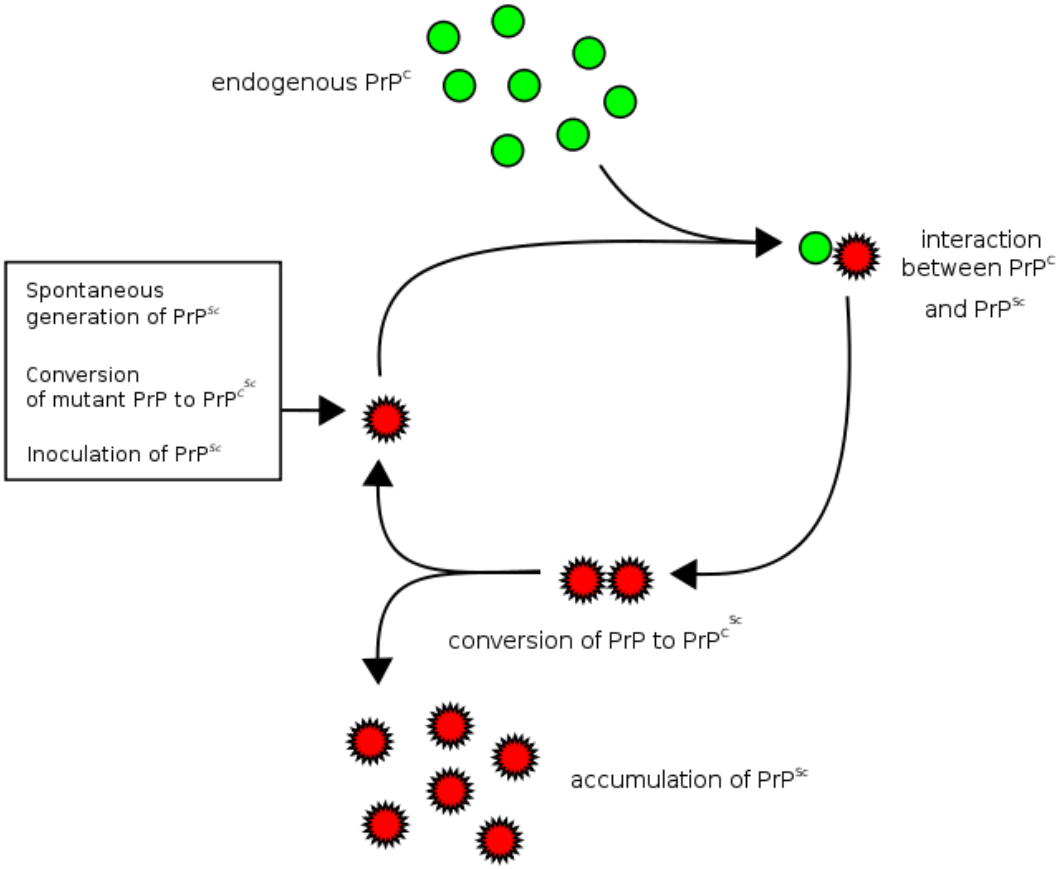
$PrP^{C}$ is a normal protein found on the membranes of cells. It has 209 amino acids (in humans), one disulfide bond, a molecular weight of 35-36 kDa and a mainly alpha-helical structure. Several topological forms exist; one cell surface form anchored via glycolipid and two transmembrane forms. The normal protein is not sedimentable; meaning it cannot be separated by centrifuging techniques. Its function is a complex issue that continues to be investigated. $PrP^{C}$ binds copper (II) ions with high affinity. The significance of this finding is not clear, but it presumably relates to PrP structure or function. $PrP^{C}$ is readily digested by proteinase K and can be liberated from the cell surface in vitro by the enzyme phosphoinositide phospholipase C (PI-PLC), which cleaves the glycophosphatidylinositol (GPI) glycolipid anchor. PrP has been reported to

play important roles in cell-cell adhesion and intracellular signaling *in vivo*, and may therefore be involved in cell-cell communication in the brain.

## $PrP^{Sc}$

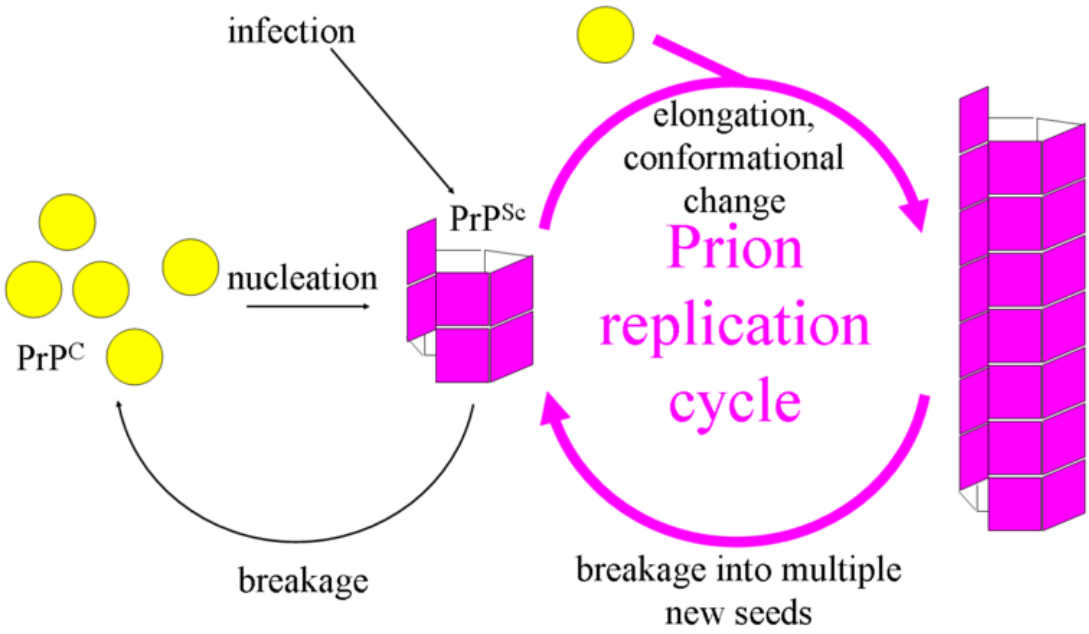
The infectious isoform of PrP, known as $PrP^{Sc}$, is able to convert normal $PrP^{C}$ proteins into the infectious isoform by changing their conformation, or shape; this, in turn, alters the way the proteins interconnect. Although the exact 3D structure of $PrP^{Sc}$ is not known, it has a higher proportion of β-sheet structure in place of the normal α-helix structure. Aggregations of these abnormal isoforms form highly structured amyloid fibers, which accumulate to form plaques. It is unclear if these aggregates are the cause of cell damage or are simply a side effect of the underlying disease process. The end of each fiber acts as a template onto which free protein molecules may attach, allowing the fiber to grow. Only PrP molecules with an identical amino acid sequence to the infectious $PrP^{Sc}$ are incorporated into the growing fiber. However, this property is not strictly shared by other proteins considered prions. The sup35p was shown to be able to be incorporated into existing aggregations even when three of the five oligopeptide repeats normally present were deleted.

## *Prion replication mechanism*




Heterodimer model of prion propagation

The first hypothesis that tried to explain how prions replicate in a protein-only manner was the **heterodimer model**. This model assumed that a single $PrP^{Sc}$ molecule binds to a single $PrP^{C}$ molecule and catalyzes its conversion into $PrP^{Sc}$. The two $PrP^{Sc}$ molecules then come apart and can go on to convert more $PrP^{C}$. However, Manfred Eigen showed that since $PrP^{C}$ has a very low rate of spontaneous conversion into $PrP^{Sc}$, the heterodimer model requires $PrP^{Sc}$ to be an extraordinarily effective catalyst, increasing the rate of the conversion reaction by a factor of around $10^{15}$. What is more, despite considerable effort, infectious monomeric $PrP^{Sc}$ has never been isolated. Theory and experiments both suggest that $PrP^{Sc}$ exists only in aggregated forms such as amyloid, and that prion replication involves cooperativity.




Prion nucleation is rare, but can be bypassed by infection. Either nucleation or infection can initiate a replication cycle of fibril growth and breakage.

An alternative model assumes that $PrP^{Sc}$ exists only as fibrils, and that fibril ends bind $PrP^{C}$ and convert it into $PrP^{Sc}$. If this were all, then the quantity of prions would increase linearly, forming ever longer fibrils. But exponential growth of both $PrP^{Sc}$ and of the quantity of infectious particles is observed during prion disease. This can be explained by taking into account fibril breakage. A mathematical solution for the exponential growth rate resulting from the combination of fibril growth and fibril breakage has been found. The exponential growth rate depends largely on the square root of the $PrP^{C}$ concentration. The incubation period is determined by the exponential growth rate, and in vivo data on prion diseases in transgenic mice match this prediction. The same square root dependence is also seen in vitro in experiments with a variety of different amyloid proteins.

The mechanism of prion replication has implications for designing drugs. Since the incubation period of prion diseases is so long, an effective drug does not need to eliminate all prions, but simply needs to slow down the rate of exponential growth. Models predict that the most effective way to achieve this, using a drug with the lowest possible dose, is to find a drug that binds to fibril ends and blocks them from growing any further.

## *PrP function*

It has been proposed that neurodegeneration caused by prions may be related to abnormal function of PrP. However, the physiological function of the prion protein remains a controversial matter. While data from in vitro experiments suggest many dissimilar roles, studies on PrP knockout mice have provided only limited information because these animals exhibit only minor abnormalities. In recent research done in mice, it was found that the cleavage of prions in peripheral nerves causes the activation of myelin repair in Schwann Cells and that the lack of prions caused demyelination in those cells.

### PrP and long-term memory

There is evidence that PrP may have a normal function in maintenance of long-term memory. Maglio and colleagues have shown that mice without the genes for normal cellular PrP protein have altered hippocampal long-term potentiation.

### PrP and stem cell renewal

A 2006 article from the Whitehead Institute for Biomedical Research indicates that PrP expression on stem cells is necessary for an organism's self-renewal of bone marrow. The study showed that all long-term hematopoietic stem cells expressed PrP on their cell membrane and that hematopoietic tissues with PrP-null stem cells exhibited increased sensitivity to cell depletion.

## *Prion disease*

Diseases caused by prions

| Affected animal(s) | Disease |
|---|---|
| sheep, goat | Scrapie |
| cattle | Bovine spongiform encephalopathy (BSE), mad cow disease |
| mink | Transmissible mink encephalopathy (TME) |
| white-tailed deer, elk, mule deer, moose | Chronic wasting disease (CWD) |
| cat | Feline spongiform encephalopathy (FSE) |
| nyala, oryx, greater kudu | Exotic ungulate encephalopathy (EUE) |
| ostrich | Spongiform encephalopathy (Not been shown to be transmissible.) |
| human | Creutzfeldt–Jakob disease |

(CJD)

iatrogenic Creutzfeldt-Jakob disease (iCJD)

variant Creutzfeldt-Jakob disease (vCJD)

familial Creutzfeldt-Jakob disease (fCJD)

sporadic Creutzfeldt-Jakob disease (sCJD)

Gerstmann–Sträussler–Scheinker syndrome (GSS)

Fatal familial insomnia (FFI)

Kuru

Prions cause neurodegenerative disease by aggregating extracellularly within the central nervous system to form plaques known as amyloid, which disrupt the normal tissue structure. This disruption is characterized by "holes" in the tissue with resultant spongy architecture due to the vacuole formation in the neurons. Other histological changes include astrogliosis and the absence of an inflammatory reaction. While the incubation period for prion diseases is generally quite long, once symptoms appear the disease progresses rapidly, leading to brain damage and death. Neurodegenerative symptoms can include convulsions, dementia, ataxia (balance and coordination dysfunction), and behavioural or personality changes.

All known prion diseases, collectively called *transmissible spongiform encephalopathies* (TSEs), are untreatable and fatal. A vaccine has been developed in mice, however, that may provide insight into providing a vaccine in humans to resist prion infections. Additionally, in 2006 scientists announced that they had genetically engineered cattle lacking a necessary gene for prion production – thus theoretically making them immune to BSE, building on research indicating that mice lacking normally occurring prion protein are resistant to infection by scrapie prion protein.

Many different mammalian species can be affected by prion diseases, as the prion protein (PrP) is very similar in all mammals. Due to small differences in PrP between different species it is unusual for a prion disease to be transmitted from one species to another. The human prion disease *variant Creutzfeldt-Jakob disease*, however, is believed to be caused by a prion which typically infects cattle, causing Bovine spongiform encephalopathy and is transmitted through infected meat.

## Transmission

It has been recognized that prion diseases can arise in three different ways: acquired, familial, or sporadic. It is often assumed that the diseased form directly interacts with the normal form to make it rearrange its structure. One idea, the "Protein X" hypothesis, is

that an as-yet unidentified cellular protein (Protein X) enables the conversion of $PrP^{C}$ to $PrP^{Sc}$ by bringing a molecule of each of the two together into a complex.

Current research suggests that the primary method of infection in animals is through ingestion. It is thought that prions may be deposited in the environment through the remains of dead animals and via urine, saliva, and other body fluids. They may then linger in the soil by binding to clay and other minerals.

A University of Californian research team, led by Nobel prize winner Stanley Prusiner, has proven that infection can occur from prions in manure. And since manure is present in many areas surrounding water reservoirs, as well as used on many crop fields, it raises the possibility of widespread transmission.

## Sterilization

Infectious particles possessing nucleic acid are dependent upon it to direct their continued replication. Prions, however, are infectious by their effect on normal versions of the protein. Sterilizing prions therefore involves the denaturation of the protein to a state where the molecule is no longer able to induce the abnormal folding of normal proteins. Prions are generally quite resistant to proteases, heat, radiation, and formalin treatments, although their infectivity can be reduced by such treatments. Effective prion decontamination relies upon protein hydrolysis or reduction or destruction of protein tertiary structure. Examples include bleach, caustic soda, and strong acidic detergents such as LpH. 134°C (274°F) for 18 minutes in a pressurized steam autoclave may not be enough to deactivate the agent of disease. Ozone sterilization is currently being studied as a potential method for prion denature and deactivation. Renaturation of a completely denatured prion to infectious status has not yet been achieved, however partially denatured prions can be renatured to an infective status under certain artificial conditions.

The World Health Organization recommends any of the following three procedures for the sterilization of all heat-resistant surgical instruments to ensure that they are not contaminated with prions:

1. Immerse in a pan containing 1N NaOH and heat in a gravity-displacement autoclave at 121°C for 30 minutes; clean; rinse in water; and then perform routine sterilization processes.
2. Immerse in 1N NaOH or sodium hypochlorite (20,000 parts per million available chlorine) for 1 hour; transfer instruments to water; heat in a gravity-displacement autoclave at 121°C for 1 hour; clean; and then perform routine sterilization processes.
3. Immerse in 1N NaOH or sodium hypochlorite (20,000 parts per million available chlorine) for 1 hour; remove and rinse in water, then transfer to an open pan and heat in a gravity-displacement (121°C) or in a porous-load (134°C) autoclave for 1 hour; clean; and then perform routine sterilization processes.

## Debate

Whether prions are the agent which causes disease or merely a symptom caused by a different agent is still debated by a minority of researchers. The following sections describe several alternative hypotheses: some pertain to the composition of the infectious agent (protein-only, protein with other components, virus, or other), while others pertain to its mechanism of reproduction.

## Protein hypothesis

Prior to the discovery of prions, it was thought that all pathogens used nucleic acids to direct their replication. The "protein hypothesis" states that a protein structure can replicate without the use of nucleic acid. This was initially controversial as it contradicts the so-called "central dogma of molecular biology", which describes nucleic acid as the central form of replicative information.

Evidence in favor of a protein hypothesis includes:

- No virus particles, bacteria, or fungi have been conclusively associated with prion diseases, although *Saccharomyces cerevisiae* has been known to be associated with infectious, yet non-lethal prions, such as Sup35p.
- No nucleic acid has been conclusively associated with infectivity; agent is resistant to ultraviolet radiation
- No immune response to infection
- $PrP^{Sc}$ experimentally transmitted between one species and another results in $PrP^{Sc}$ with the amino-acid sequence of the recipient species, suggesting that replication of the donor agent does not occur
- Familial prion disease occurs in families with a mutation in the PrP gene, and mice with PrP mutations develop prion disease despite controlled conditions where transmission is prevented
- Animals lacking $PrP^{C}$ do not contract prion disease.
- Infectious prions can be formed *de novo* from purified non-infectious components, in the absence of gene-coding nucleic acids.

## Genetic factors

A gene for the normal protein has been identified: the *PRNP* gene. In all inherited cases of prion disease, there is a mutation in the *PRNP* gene. Many different *PRNP* mutations have been identified and it is thought that the mutations somehow make $PrP^{C}$ more likely to change spontaneously into the abnormal $PrP^{Sc}$ form. Although this discovery puts a hole in the general prion hypothesis, that prions can only aggregate proteins of identical amino acid make up. These mutations can occur throughout the gene. Some mutations involve expansion of the octapeptide repeat region at the N-terminal of PrP. Other mutations that have been identified as a cause of inherited prion disease occur at positions 102, 117 & 198 (GSS), 178, 200, 210 & 232 (CJD) and 178 (Fatal Familial Insomnia, FFI). The cause of prion disease can be sporadic, genetic, and infectious, or a

combination of these factors. For example, in order to have scrapie, both an infectious agent and a susceptible genotype need to be present.

## Multi-component hypothesis

In 2007, biochemist Surachai Supattapone and his colleagues at Dartmouth College produced purified infectious prions *de novo* from defined components ($PrP^C$, co-purified lipids, and a synthetic polyanionic molecule). These researchers also showed that the polyanionic molecule required for prion formation was selectively incorporated into high-affinity complexes with PrP molecules, leading them to hypothesize that infectious prions may be composed of multiple host components, including PrP, lipid, and polyanionic molecules, rather than $PrP^{Sc}$ alone.

In 2010, Jiyan Ma and colleagues at The Ohio State University produced infectious prions from a recipe of bacterially expressed recombinant PrP, POPG phospholipid, and RNA, further supporting the multi-component hypothesis. This finding is in contrast to studies that found minimal infectious prions produced from recombinant PrP alone.

## Heavy metal poisoning hypothesis

Recent reports suggest that imbalance of brain metal homeostasis is a significant cause of $PrP^{Sc}$-associated neurotoxicity, though the underlying mechanisms are difficult to explain based on existing information. Proposed hypotheses include a functional role for $PrP^C$ in metal metabolism, and loss of this function due to aggregation to the disease associated $PrP^{Sc}$ form as the cause of brain metal imbalance. Other views suggest gain of toxic function by $PrP^{Sc}$ due to sequestration of $PrP^C$-associated metals within the aggregates, resulting in the generation of redox-active $PrP^{Sc}$ complexes. The physiological implications of some $PrP^C$-metal interactions are known, while others are still unclear. The pathological implications of $PrP^C$-metal interaction include metal-induced oxidative damage, and in some instances conversion of $PrP^C$ to a $PrP^{Sc}$-like form.

## Viral hypothesis

The protein-only hypothesis has been criticised by those who feel that the simplest explanation of the evidence to date is viral. For more than a decade, Yale University neuropathologist Laura Manuelidis has been proposing that prion diseases are caused instead by an unidentified slow virus. In January 2007, she and her colleagues published an article reporting to have found a virus in 10%, or less, of their scrapie-infected cells in culture.

The virion hypothesis states that TSEs are caused by a replicable informational molecule (which is likely to be a nucleic acid) bound to PrP. Many TSEs, including scrapie and BSE, show strains with specific and distinct biological properties, a feature which supporters of the virion hypothesis feel is not explained by prions.

Evidence in favor of a viral hypothesis includes:

- Strain variation: differences in prion infectivity, incubation, symptomology and progression among species resembles that seen between viruses, especially RNA viruses
- The long incubation and rapid onset of symptoms resembles lentiviruses, such as HIV-induced AIDS
- Viral-like particles that do not appear to be composed of PrP have been found in some of the cells of scrapie- or CJD-infected cell lines.

Recent studies propagating TSE infectivity in cell-free reactions and in purified component chemical reactions strongly suggest against TSE viral nature. More recently, using a similar defined recipe of multiple components (PrP, POPG lipid, RNA), Jiyan Ma and colleagues generated infectious prions from recombinant PrP expressed from *E. coli*, casting further doubt on the viral hypothesis.

## *Fungi*

Fungal proteins exhibiting templated conformational change were discovered in the yeast *Saccharomyces cerevisiae* by Reed Wickner in the early 1990s. For their mechanistic similarity to the prion hypothesis, they were termed yeast prions. Subsequently, a prion has also been found in the fungus *Podospora anserina*. These prions behave similarly to PrP, but are generally non-toxic to their hosts. Susan Lindquist's group at the Whitehead Institute has argued that some of the fungal prions are not associated with any disease state, but may have a useful role; however, researchers at the NIH have also provided arguments suggesting that fungal prions could be considered a diseased state. Thus, the issue of whether fungal proteins are diseases, or have evolved for some specific functions still remains unresolved.

As of 2010, there are 8 known prion proteins in fungi, 7 in *Saccharomyces cerevisiae* (Sup35, Rnq1, Ure2, Swi1, Mca1, Mot3, Cyc8) and one in *Podospora anserina* (HET-s).

Research into fungal prions has given strong support to the protein-only concept, since it has been demonstrated that purified protein extracted from cells with a prion state can convert the normal form of the protein into a misfolded form *in vitro*, and in the process, preserve the information corresponding to different strains of the prion state. It has also shed some light on prion domains, which are regions in a protein that promote the conversion into a prion. Fungal prions have helped to suggest mechanisms of conversion that may apply to all prions, though mammalian prions may operate by an independent mechanism. For example, mammalian prions lack characteristic fungal prion domains.

**Fungal Prions**

| Protein | Natural Host | Normal Function | Prion State | Prion Phenotype | Year Identified |
|---|---|---|---|---|---|
| Ure2p | Saccharomyces cerevisiae | Nitrogen catabolite repressor | [URE3] | Growth on poor nitrogen sources | 1994 |
| Sup35p | Saccharomyces cerevisiae | Translation termination factor | [PSI+] | Increased levels of nonsense suppression | 1994 |
| HET-S | Podospora anserina | Regulates heterokaryon incompatibility | [Het-s] | Heterokaryon formation between incompatible strains | |
| Rnq1p | Saccharomyces cerevisiae | Protein template factor | [RNQ+],[PIN+] | Promotes aggregation of other prions | |
| Mca1 | Saccharomyces cerevisiae | Putative Yeast Caspase | [MCA+] | Unknown | 2008 |
| Swi1 | Saccharomyces cerevisiae | chromatin remodeling | [SWI+] | poor growth on some carbon sources | 2008 |
| Cyc8 | Saccharomyces cerevisiae | transcriptional repressor | [OCT+] | transcriptional derepression of multiple genes | 2009 |
| Mot3 | Saccharomyces cerevisiae | Nuclear transcription factor | [MOT3+] | transcriptional derepression of anaerobic genes | 2009 |

- A putative prion protein, forming the [ISP+] element remains to be identified.

## *Potential treatments*

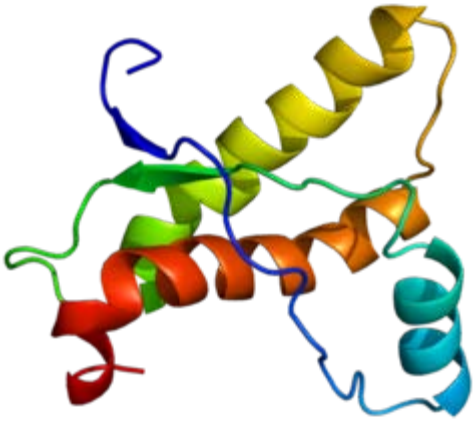
Advancements in computer modeling have allowed for scientists to identify compounds which can serve as a treatment for prion caused diseases, such as one compound found to bind a cavity in the $PrP^{C}$ and stabilize the conformation, reducing the amount of harmful $PrP^{Sc}$.

Recently, anti-prion antibodies capable of crossing the blood-brain-barrier and targeting cytosolic prion protein (an otherwise major obstacle in prion therapeutics) have been described.

## Chapter 2

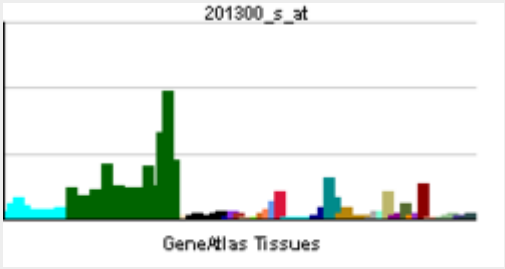
# PRNP

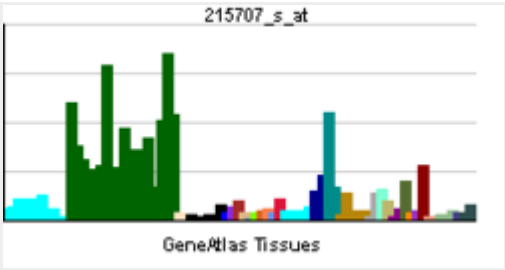
**Prion protein (p27-30) (Creutzfeldt-Jakob disease, Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia)**



PDB rendering based on 1ag2.

| Identifiers | |
|---|---|
| **Symbols** | PRNP; GSS; ASCR; CD230; CJD; MGC26679; PRIP; PrP; PrP27-30; PrP33-35C; PrPc |
| **External IDs** | OMIM: 176640 MGI: 97769 HomoloGene: 7904 GeneCards: PRNP Gene |
| **RNA expression pattern** | |



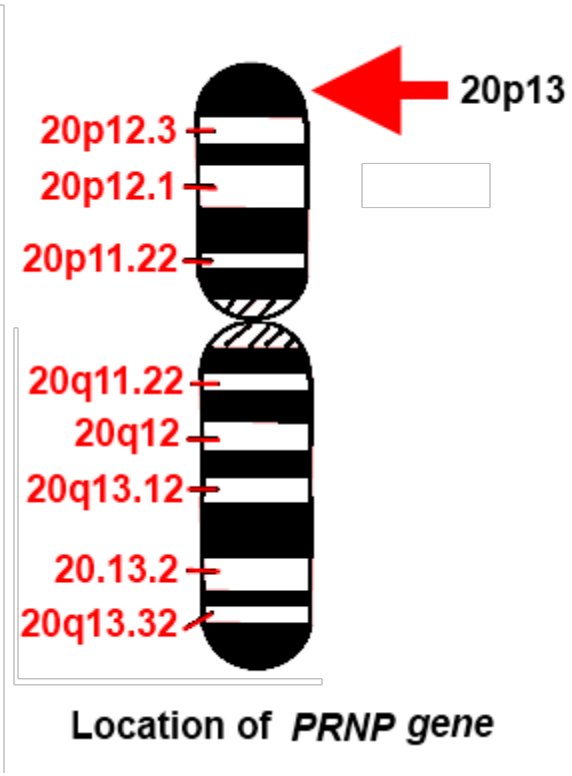




**Orthologs**

| Species | Human | Mouse |
|---|---|---|
| Entrez | 5621 | 19122 |
| Ensembl | ENSG00000171867 | n/a |
| UniProt | P04156 | n/a |
| RefSeq (mRNA) | NM_000311 | NM_011170 |
| RefSeq (protein) | NP_000302 | NP_035300 |
| Location (UCSC) | Chr 20: 4.61 - 4.63 Mb | n/a |
| PubMed search | | |

**Major prion protein** (PrP) also known as **CD230** (cluster of differentiation 230) is a protein that in humans is encoded by the ***PRNP*** gene (PRioN Protein (Creutzfeld-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia)). The major prion protein is expressed in the brain and several other tissues.

The human PRNP gene is located on the short (p) arm of chromosome 20 between the end (terminus) of the arm and position 12, from base pair 4,615,068 to base pair 4,630,233.



Location of *PRNP gene*

## *Structure and function*

Two distinct major conformational forms of PrP have been identified in the nervous system. The usual cellular form is denoted $PrP^{C}$ (for ***c**ellular* or ***c**ommon* form). Another form, $PrP^{Sc}$ (for ***sc**rapie*), has an identical amino acid sequence, but is folded into a different shape or conformation. The $PrP^{Sc}$ form is associated with a number of degenerative disorders of the brain and nervous system. In a process that is not fully understood, $PrP^{C}$ is occasionally transformed into the infectious isomer $PrP^{Sc}$. This abnormal protein can further promote the transformation of $PrP^{C}$ into $PrP^{Sc}$, leading to transmissible spongiform encephalopathy.

The primary sequence of PrP is 253 amino acids long before posttranslational modification. Signal sequences in the amino- and carboxy- terminal ends which are removed posttranslationally. For human and Syrian hamster PrP, two glycosylated sites exist on helices 2 and 3 at Asn181 and Asn197. Murine PrP has glycosylation sites as Asn180 and Asn196. A disulfide bond exists between Cys179 of the second helix and Cys214 of the third helix (human $PrP^{C}$ numbering).

## $PrP^{C}$ (normal cellular) isoform

Although the precise function of PrP is not yet known, it is possibly involved in the transport of ionic copper to cells from the surrounding environment. Researchers have also proposed roles for PrP in cell signaling or in the formation of synapses . $PrP^{C}$

attaches to the outer surface of the cell membrane by a glycosylphosphatidylinositol anchor at its C-terminal Ser231.

Prion protein contains 5 amino-terminal octapeptide repeats with sequence PHGGGWGQ. This is thought to generate a calcium binding domain via nitrogen atoms in the histidine imidazole side chains and deprotonated amide nitrogens from the 2nd and 3rd glycines in the repeat. The ability to bind calcium is therefore pH dependent. NMR shows calcium binding results in a conformational change at the N-terminus.

### $PrP^{Sc}$ (scrapie) isoform

The abnormal $PrP^{Sc}$ isoform has a different secondary and tertiary structure from $PrP^{C}$, but identical primary sequence. Circular dichroism shows that normal $PrP^{C}$ had 43% alpha helical and 3% beta sheet content, whilst $PrP^{Sc}$ was only 30% alpha helix and 43% beta sheet. This refolding renders the $PrP^{Sc}$ isoform extremely resistant to proteolysis.

Polymorphisms at sites 136, 154 and 171 are associated with varying susceptibility to scrapie. Polymorphisms of the PrP-VRQ form and PrP-ARQ form are associated with increased susceptibility, whilst PrP-ARR is associated with resistance.

The National Scrapie Plan aims to breed out these scrapie polymorphisms by increasing the frequency of the resistant allele. However, PrP-ARR polymorphisms are susceptible to atypical scrapie so this may prove unfruitful.

## *Diseases caused by PrP misfolding*

More than 20 mutations in the PRNP gene have been identified in people with inherited prion diseases, which include the following:

- Creutzfeldt-Jakob disease - aspartic acid-178 is replaced by asparagine while valine is present at amino acid 129
- Gerstmann-Sträussler-Scheinker syndrome - usually a change in codon 102 from proline to leucine
- fatal familial insomnia - aspartic acid-178 is replaced by asparagine while methionine is present at amino acid 129

Some PRNP mutations lead to a change in single amino acids (the building blocks of proteins) in the prion protein. Others insert additional amino acids into the protein or cause an abnormally short protein to be made. These mutations cause the cell to make prion proteins with an abnormal structure. The abnormal protein, $PrP^{Sc}$, accumulates in the brain and destroys nerve cells, which leads to the mental and behavioral features of prion diseases.

Several other changes in the PRNP gene (called polymorphisms) do not cause prion diseases, but may affect a person's risk of developing these diseases or alter the course of

the disorders. An allele which codes for a PRNP variant — G127V provides resistance to Kuru.

## *Interactions*

PRNP has been shown to interact with Hop.

## Chapter 3

# Fungal Prions

**Fungal prions** provide an excellent model for the understanding of disease-forming mammalian prions. Fungal prions are naturally occurring proteins that can undergo a structural conversion that becomes self-propagating and infectious. They represent an epigenetic phenomenon in which information is not encoded in the nuclear DNA, but is structurally encoded within the protein. Several prion-forming proteins have been identified in fungi, primarily in the yeast Saccharomyces cerevisiae. Some of these are not associated with any disease state and may possibly have a beneficial role by giving an evolutionary advantage to their host.

## *The HET-s Prion of* Podospora anserina

*Podospora anserina* is a filamentous fungus. Genetically compatible colonies of this fungus can merge together and share cellular contents such as nutrients and cytoplasm. A natural system of protective "incompatibility" proteins exists to prevent promiscuous sharing between unrelated colonies. One such protein, called HET-S, adopts a prion-like form in order to function properly. The prion form of HET-S spreads rapidly throughout the cellular network of a colony and can convert the non-prion form of the protein to a prion state after compatible colonies have merged. However, when an incompatible colony tries to merge with a prion-containing colony, the prion causes the "invader" cells to die, ensuring that only related colonies obtain the benefit of sharing resources.

## *Prions of Yeast*

### [PSI+] & [URE3]

In 1965, Brian Cox, a geneticist working with the yeast *Saccharomyces cerevisiae*, described a genetic trait (termed [PSI+]) with an unusual pattern of inheritance. The initial discovery of [PSI+] was made in a strain auxotrophic for adenine due to a nonsense mutation. Despite many years of effort, Cox could not identify a conventional

mutation that was responsible for the [PSI+] trait. In 1994, yeast geneticist Reed Wickner correctly hypothesized that [PSI+] as well as another mysterious heritable trait, [URE3], resulted from prion forms of certain normal cellular proteins. The names of yeast prions are frequently placed within brackets to indicate that they are non-mendelian in their passage to progeny cells, much like plasmid and mitochondrial DNA.

It was soon noticed that heat shock proteins (which help other proteins fold properly) such as Hsp104 were intimately tied to the inheritance and transmission of [PSI+] and many other yeast prions. Since then, researchers have unravelled how the proteins that code for [PSI+] and [URE3] can convert between prion and non-prion forms, as well as the consequences of having intracellular prions.

When exposed to certain adverse conditions, in some genetic backgrounds [PSI+] cells actually fare better than their prion-free siblings; this finding suggests that the ability to adopt a [PSI+] prion form may result from positive evolutionary selection. It has been speculated that the ability to convert between prion-infected and prion-free forms acts as an evolutionary capacitor to enable yeast to quickly and reversibly adapt in variable environments. Nevertheless, Wickner maintains that URE3 and [PSI+] are diseases, although this claim has been challenged using theoretical population genetic models.

Further investigation found that [PSI+] is the result of a self-propagating misfolded form of Sup35p, which is an important factor for translation termination during protein synthesis. In [PSI+] yeast cells the Sup35 protein forms filamentous aggregates known as amyloid. The amyloid conformation is self-propagating and represents the prion state. It is believed that suppression of nonsense mutations in [PSI+] cells is due to a reduced amount of functional Sup35 because much of the protein is in the amyloid state. The Sup35 protein assembles into amyloid via an amino-terminal prion domain. The structure is based on the stacking of the prion domains in an in-register and parallel beta sheet confirmation.

Laboratories commonly identify [PSI+] by growth of a strain auxotrophic for adenine on media lacking adenine, similar to that used by Cox et al. These strains cannot synthesize adenine due to a nonsense mutation in one of the enzymes involved in biosynthetic pathway. When the strain is grown on yeast-extract/dextrose/peptone media (YPD), the blocked pathway results in buildup of a red-colored intermediate compound, which is exported from the cell due to its toxicity. Hence, color is an alternative method of identifying [PSI+] -- [PSI+] strains are white or pinkish in color, and [psi-] strains are red. A third method of identifying [PSI+] is by the presence of Sup35 in the pelleted fraction of cellular lysate.

## [PIN+]

[PIN+], in turn, is the misfolded form of the protein Rnq1. However, the normal function of this protein is unknown to date. It is of note that for the induction of most variants of [PSI+], the presence of [PIN+] is required. Though reasons for this are poorly understood, it is suggested that [PIN+] aggregates may act as “seeds” for the

polymerization of [PSI+]. Like Sup35 and Ure2, the basis of the [PIN+] prion is an amyloid form of Rnq1. The amyloid is composed of the Rnq1 protein arranged in in-register parallel beta sheets, like the amyloid form of Sup35. Due to similar amyloid structures, the [PIN+] prion may facilitate the formation of [PSI+] through a templating mechanism.

Two modified versions of Sup35 have been created that can induce PSI+ in the absence of [PIN+] when overexpressed. One version was created by digestion of the gene with BalI, which results in a protein consisting of only the M and N portions of Sup35. The other is a fusion of Sup35NM with HPR, a human membrane receptor protein.

## *List of Characterized Fungal Prions*

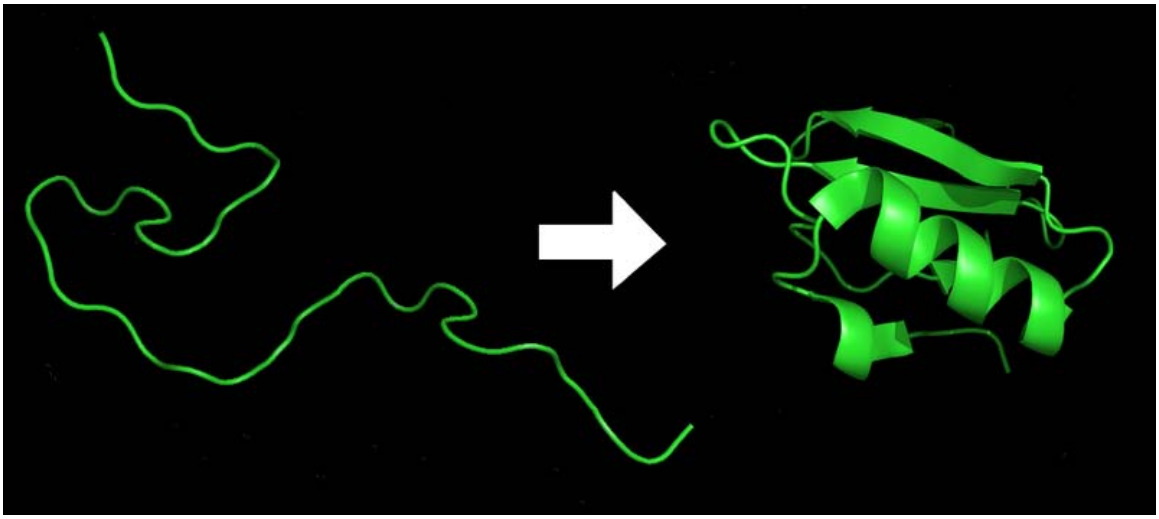
**Fungal Prions**

| Protein | Natural Host | Normal Function | Prion State | Prion Phenotype | Year Identified |
|---|---|---|---|---|---|
| Ure2p | *Saccharomyces cerevisiae* | Nitrogen catabolite repressor | [URE3] | Growth on poor nitrogen sources | 1994 |
| Sup35p | *Saccharomyces cerevisiae* | Translation termination factor | [PSI+] | Increased levels of nonsense suppression | 1994 |
| HET-S | *Podospora anserina* | Regulates heterokaryon incompatibility | [Het-s] | Heterokaryon formation between incompatible strains | |
| Rnq1p | *Saccharomyces cerevisiae* | Protein template factor | [RNQ+],[PIN+] | Promotes aggregation of other prions | |
| Mca1 | *Saccharomyces cerevisiae* | Putative Yeast Caspase | [MCA+] | Unknown | 2008 |
| Swi1 | *Saccharomyces cerevisiae* | chromatin remodeling | [SWI+] | poor growth on some carbon sources | 2008 |
| Cyc8 | *Saccharomyces cerevisiae* | transcriptional repressor | [OCT+] | transcriptional derepression of multiple genes | 2009 |
| Mot3 | *Saccharomyces cerevisiae* | nuclear transcription factor | [MOT3+] | transcriptional derepression of anaerobic genes | 2009 |
| Pma1 the major plasma membrane | *Saccharomyces cerevisiae* | | [GAR+] | resistant to glucose-associated repression | 2009 |

| | | | | | |
|---|---|---|---|---|---|
| proton pump Std1 | | | | | |
| Sfp1 | *Saccharomyces cerevisiae* | global transcriptional regulator | [ISP+] | antisuppressor of certain *sup35* mutations | 2010 |

## Chapter 4

# Protein Folding



Protein before and after folding.

**Protein folding** is the physical process by which a polypeptide folds into its characteristic and functional three-dimensional structure from random coil. Each protein exists as an unfolded polypeptide or random coil when translated from a sequence of mRNA to a linear chain of amino acids. This polypeptide lacks any developed three-dimensional structure (the left hand side of the neighboring figure). Amino acids interact with each other to produce a well-defined three-dimensional structure, the folded protein (the right hand side of the figure), known as the native state. The resulting three-dimensional structure is determined by the amino acid sequence (Anfinsen's dogma).

The correct three-dimensional structure is essential to function, although some parts of functional proteins may remain unfolded Failure to fold into the intended shape usually produces inactive proteins with different properties including toxic prions. Several neurodegenerative and other diseases are believed to result from the accumulation of *misfolded* (incorrectly folded) proteins. Many allergies are caused by the folding of the

proteins, for the immune system does not produce antibodies for certain protein structures.

## *Known facts*

### Relationship between folding and amino acid sequence

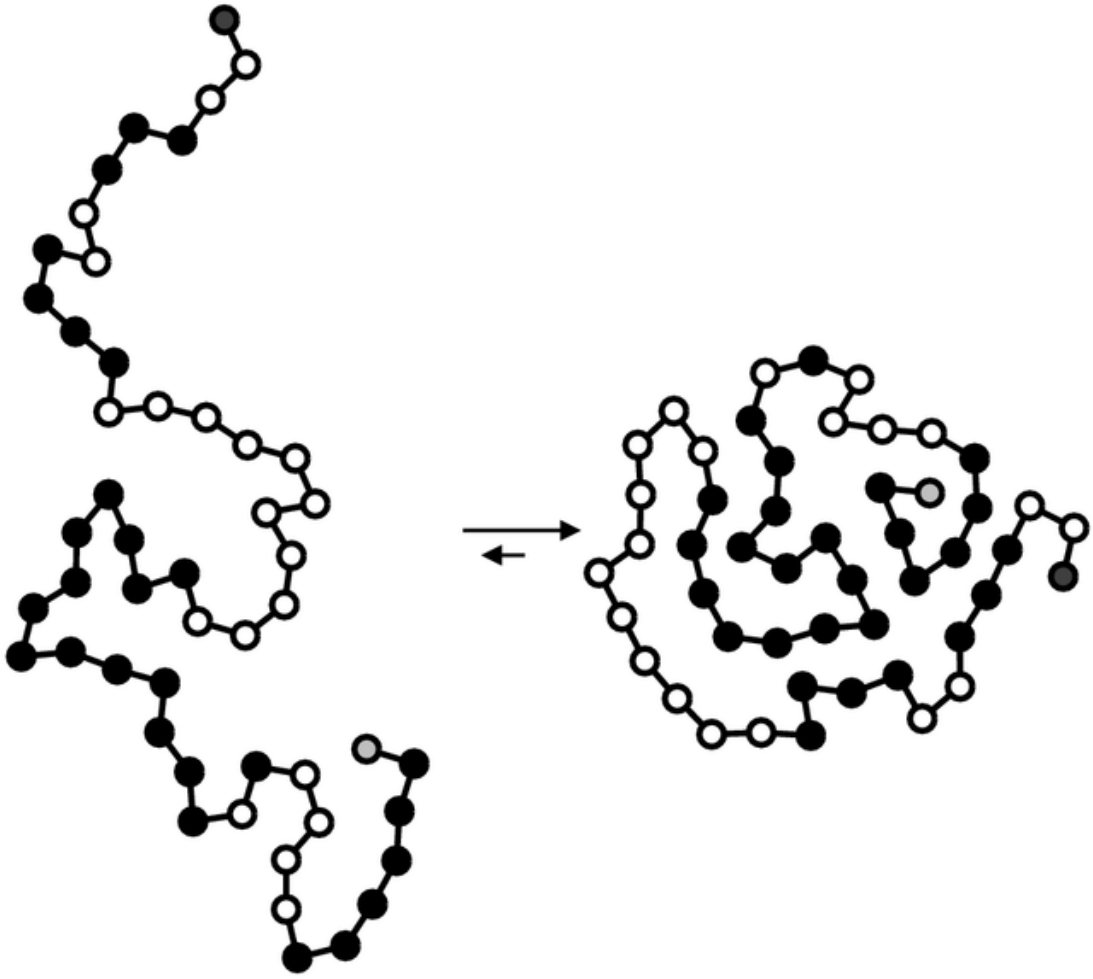


Illustration of the main driving force behind protein structure formation. In the compact fold (to the right), the hydrophobic amino acids (shown as black spheres) are in general shielded from the solvent.

The amino-acid sequence (or primary structure) of a protein determines its native conformation. A protein molecule folds spontaneously during or after biosynthesis. While these macromolecules may be regarded as "folding themselves", the process also depends on the solvent (water or lipid bilayer), the concentration of salts, the temperature, and the presence of molecular chaperones.

Folded proteins usually have a hydrophobic core in which side chain packing stabilizes the folded state, and charged or polar side chains occupy the solvent-exposed surface where they interact with surrounding water. Minimizing the number of hydrophobic side-chains exposed to water is an important driving force behind the folding process. Formation of intramolecular hydrogen bonds provides another important contribution to protein stability. The strength of hydrogen bonds depends on their environment, thus H-bonds enveloped in a hydrophobic core contribute more than H-bonds exposed to the aqueous environment to the stability of the native state.

The process of folding *in vivo* often begins co-translationally, so that the N-terminus of the protein begins to fold while the C-terminal portion of the protein is still being synthesized by the ribosome. Specialized proteins called chaperones assist in the folding of other proteins. A well studied example is the bacterial GroEL system, which assists in the folding of globular proteins. In eukaryotic organisms chaperones are known as heat shock proteins. Although most globular proteins are able to assume their native state unassisted, chaperone-assisted folding is often necessary in the crowded intracellular environment to prevent aggregation; chaperones are also used to prevent misfolding and aggregation which may occur as a consequence of exposure to heat or other changes in the cellular environment.

There are two models of protein folding that are currently being confirmed. The first is the diffusion collision model in which a nucleus is formed, then the secondary structure, and finally these secondary structures are collided together and pack tightly together. The next model is the nucleation-condensation model, in which the secondary and tertiary structure of the protein is made at the same time. Finally, recent studies have shown that some proteins show characteristics of both of these folding models.

For the most part, scientists have been able to study many identical molecules folding together *en masse*. At the coarsest level, it appears that in transitioning to the native state, a given amino acid sequence takes on roughly the same route and proceeds through roughly the same intermediates and transition states. Often folding involves first the establishment of regular secondary and supersecondary structures, particularly alpha helices and beta sheets, and afterwards tertiary structure. Formation of quaternary structure usually involves the "assembly" or "coassembly" of subunits that have already folded. The regular alpha helix and beta sheet structures fold rapidly because they are stabilized by intramolecular hydrogen bonds, as was first characterized by Linus Pauling. Protein folding may involve covalent bonding in the form of disulfide bridges formed between two cysteine residues or the formation of metal clusters. Shortly before settling into their more energetically favourable native conformation, molecules may pass through an intermediate "molten globule" state.

The essential fact of folding, however, remains that the amino acid sequence of each protein contains the information that specifies both the native structure and the pathway to attain that state. This is not to say that nearly identical amino acid sequences always fold similarly. Conformations differ based on environmental factors as well; similar proteins fold differently based on where they are found. Folding is a spontaneous process

independent of energy inputs from nucleoside triphosphates. The passage of the folded state is mainly guided by hydrophobic interactions, formation of intramolecular hydrogen bonds, and van der Waals forces, and it is opposed by conformational entropy.

## Disruption of the native state

Under some conditions proteins will not fold into their biochemically functional forms. Temperatures above or below the range that cells tend to live in will cause thermally unstable proteins to unfold or "denature" (this is why boiling makes an egg white turn opaque). High concentrations of solutes, extremes of pH, mechanical forces, and the presence of chemical denaturants can do the same. Protein thermal stability is far from constant, however. For example, hyperthermophilic bacteria have been found that grow at temperatures as high as 122 °C, which of course requires that their full complement of vital proteins and protein assemblies be stable at that temperature or above.

A fully denatured protein lacks both tertiary and secondary structure, and exists as a so-called random coil. Under certain conditions some proteins can refold; however, in many cases denaturation is irreversible. Cells sometimes protect their proteins against the denaturing influence of heat with enzymes known as chaperones or heat shock proteins, which assist other proteins both in folding and in remaining folded. Some proteins never fold in cells at all except with the assistance of chaperone molecules, which either isolate individual proteins so that their folding is not interrupted by interactions with other proteins or help to unfold misfolded proteins, giving them a second chance to refold properly. This function is crucial to prevent the risk of precipitation into insoluble amorphous aggregates.

## Incorrect protein folding and neurodegenerative disease

Aggregated proteins are associated with prion-related illnesses such as Creutzfeldt-Jakob disease, bovine spongiform encephalopathy (mad cow disease), amyloid-related illnesses such as Alzheimer's disease and familial amyloid cardiomyopathy or polyneuropathy, as well as intracytoplasmic aggregation diseases such as Huntington's and Parkinson's disease. These age onset degenerative diseases are associated with the multimerization of misfolded proteins into insoluble, extracellular aggregates and/or intracellular inclusions including cross-beta sheet amyloid fibrils; it is not clear whether the aggregates are the cause or merely a reflection of the loss of protein homeostasis, the balance between synthesis, folding, aggregation and protein turnover. Misfolding and excessive degradation instead of folding and function leads to a number of proteopathy diseases such as antitrypsin-associated emphysema, cystic fibrosis and the lysosomal storage diseases, where loss of function is the origin of the disorder. While protein replacement therapy has historically been used to correct the latter disorders, an emerging approach is to use pharmaceutical chaperones to fold mutated proteins to render them functional.

## Effect of external factors on the folding of Proteins

Several external factors such as temperature, external fields (electric, magnetic), , molecular crowding , limitation of space could have a big influence on the folding of proteins . Modification of the local minima by external factors can also induce modifications of the folding trajectory.

## The Levinthal paradox and kinetics

The Levinthal paradox observes that if a protein were to fold by sequentially sampling all possible conformations, it would take an astronomical amount of time to do so, even if the conformations were sampled at a rapid rate (on the nanosecond or picosecond scale). Based upon the observation that proteins fold much faster than this, Levinthal then proposed that a random conformational search does not occur, and the protein must, therefore, fold through a series of meta-stable intermediate states.

The duration of the folding process varies dramatically depending on the protein of interest. When studied outside the cell, the slowest folding proteins require many minutes or hours to fold primarily due to proline isomerization, and must pass through a number of intermediate states, like checkpoints, before the process is complete. On the other hand, very small single-domain proteins with lengths of up to a hundred amino acids typically fold in a single step. Time scales of milliseconds are the norm and the very fastest known protein folding reactions are complete within a few microseconds.

## *Energy landscape theory of protein folding*

The protein folding phenomenon was largely an experimental endeavor until the formulation of an energy landscape theory of proteins by Joseph Bryngelson and Peter Wolynes in the late 1980s and early 1990s. This approach introduced the *principle of minimal frustration*,. This principle says that nature has chosen amino acid sequences so that the folded state of the protein is very stable. Additionally, the undesired interactions between amino acids along the folding pathway are reduced making the acquisition of the folded state a very fast process. Even though nature has reduced the level of *frustration* in proteins, some degree of it remains up to now as can be observed in the presence of local minima in the energy landscape of proteins. A consequence of these evolutionarily selected sequences is that proteins are generally thought to have globally "funneled energy landscapes" (coined by José Onuchic) that are largely directed towards the native state. This "folding funnel" landscape allows the protein to fold to the native state through any of a large number of pathways and intermediates, rather than being restricted to a single mechanism. The theory is supported by both computational simulations of model proteins and experimental studies, and it has been used to improve methods for protein structure prediction and design. The description of protein folding by the leveling free-energy landscape is also consistent with the $2^{nd}$ law of thermodynamics. Physically, thinking of landscapes in terms of visualizable potential or total energy surfaces simply with maxima, saddle points, minima and funnels, rather like geographic landscapes, is perhaps a little misleading.

## *Techniques for studying protein folding*

### Circular dichroism

Circular dichroism is one of the most general and basic tools to study protein folding. Circular dichroism spectroscopy measures the absorption of circularly polarized light. In proteins, structures such as alpha helicies and beta sheets are chiral, and thus absorb such light. The absorption of this light acts as a marker of the degree of foldedness of the protein ensemble. This technique can be used to measure equilibrium unfolding of the protein by measuring the change in this absorption as a function of denaturant concentration or temperature. A denaturant melt measures the free energy of unfolding as well as the protein's m value, or denaturant dependence. A temperature melt measures the melting temperature ($T_m$) of the protein. This type of spectroscopy can also be combined with fast-mixing devices, such as stopped flow, to measure protein folding kinetics and to generate chevron plots.

### Dual Polarisation Interferometry

Dual polarisation interferometry is a surface based technique for measuring the optical properties of molecular layers. When used to characterise protein folding, it measures the conformation by determining the overall size of a monolayer of the protein and its density in real time at sub-Angstrom resolution. Although real time, measurement of the kinetics of protein folding are limited to processes that occur slower than ~10 Hz. Similar to circular dichroism the stimulus for folding can be a denaturant or temperature.

### Vibrational circular dichroism of proteins

The more recent developments of vibrational circular dichroism (VCD) techniques for proteins, currently involving Fourier transform (FFT) instruments, provide powerful means for determining protein conformations in solution even for very large protein molecules. Such VCD studies of proteins are often combined with X-ray diffraction of protein crystals, FT-IR data for protein solutions in heavy water ($D_2O$), or *ab initio* quantum computations to provide unambiguous structural assignments that are unobtainable from CD.

### Modern studies of folding with high time resolution

The study of protein folding has been greatly advanced in recent years by the development of fast, time-resolved techniques. These are experimental methods for rapidly triggering the folding of a sample of unfolded protein, and then observing the resulting dynamics. Fast techniques in widespread use include neutron scattering, ultrafast mixing of solutions, photochemical methods, and laser temperature jump spectroscopy. Among the many scientists who have contributed to the development of these techniques are Jeremy Cook, Heinrich Roder, Harry Gray, Martin Gruebele, Brian Dyer, William Eaton, Sheena Radford, Chris Dobson, Alan Fersht, Bengt Nölting and Lars Konermann.

## Computational prediction of protein tertiary structure

*De novo* or *ab initio* techniques for computational protein structure prediction are related to, but strictly distinct from, studies involving protein folding. Molecular Dynamics (MD) is an important tool for studying protein folding and dynamics in silico. Because of computational cost, ab initio MD folding simulations with explicit water are limited to peptides and very small proteins. MD simulations of larger proteins remain restricted to dynamics of the experimental structure or its high-temperature unfolding. In order to simulate long time folding processes (beyond about 1 microsecond), like folding of small-size proteins (about 50 residues) or larger, some approximations or simplifications in protein models need to be introduced. An approach using reduced protein representation (pseudo-atoms representing groups of atoms are defined) and statistical potential is not only useful in protein structure prediction, but is also capable of reproducing the folding pathways.

There are distributed computing projects which use idle CPU or GPU time of personal computers to solve problems such as protein folding or prediction of protein structure. One such prominent example being the Folding@Home project. People can run these programs on their computer or PlayStation 3 to support them.

## Experimental techniques of protein structure determination

Folded structures of proteins are routinely determined by X-ray crystallography and NMR. Dynamic methods to characterise protein folding such as dual polarisation interferomety and CD provide a measurement of conformation and conformational change rather than structure.

## Chapter 5

# Transmissible Spongiform Encephalopathy

| Transmissible spongiform encephalopathy | |
|---|---|
| **ICD-10** | A81. |
| **ICD-9** | 046 |
| **DiseasesDB** | 25165 |
| **eMedicine** | neuro/662 |
| **MeSH** | D017096 |

**Transmissible spongiform encephalopathies** (**TSE**s), also known as **prion diseases**, are a group of progressive conditions that affect the brain and nervous system of many animals, including humans. According to the most widespread hypothesis they are transmitted by prions, though some other data suggest an involvement of a *Spiroplasma* infection. Mental and physical abilities deteriorate and myriad tiny holes appear in the cortex causing it to appear like a sponge (hence 'spongiform') when brain tissue obtained at autopsy is examined under a microscope. The disorders cause impairment of brain function, including memory changes, personality changes and problems with movement that worsen over time. Prion diseases of humans include classic Creutzfeldt–Jakob disease, new variant Creutzfeldt–Jakob disease (nvCJD, a human disorder related to mad cow disease), Gerstmann–Sträussler–Scheinker syndrome, fatal familial insomnia and kuru. These conditions form a spectrum of diseases with overlapping signs and symptoms.

Unlike other kinds of infectious disease which are spread by microbes, the infectious agent in TSEs is a specific protein called prion protein. Misshaped prion proteins carry the disease between individuals and cause deterioration of the brain. TSEs are unique diseases in that their aetiology may be genetic, sporadic or infectious via ingestion of infected foodstuffs and via iatrogenic means (e.g. blood transfusion). Most TSEs are sporadic and occur in an animal with no prion protein mutation. Inherited TSE occurs in

animals carrying a rare mutant prion allele, which expresses prion proteins that contort by themselves into the disease-causing conformation. Transmission occurs when healthy animals consume tainted tissues from others with the disease. In recent times a type of TSE called bovine spongiform encephalopathy (BSE) spread in cattle in an epidemic fashion. This occurred because cattle were fed the processed remains of other cattle, a practice now banned in many countries. The epidemic could have begun with just one cow with sporadic disease.

Prions cannot be transmitted through the air or through touching or most other forms of casual contact. However, they may be transmitted through contact with infected tissue, body fluids, or contaminated medical instruments. Normal sterilization procedures such as boiling or irradiating materials fail to render prions non-infective.

## Classification

Known spongiform encephalopathies

| ICTVdb Code | Disease name | Natural host | Prion name | PrP isoform |
|---|---|---|---|---|
| | **Non-human mammals** | | | |
| 90.001.0.01.001. | Scrapie | Sheep and goats | Scrapie prion | $OvPrP^{Sc}$ |
| 90.001.0.01.002. | Transmissible mink encephalopathy (TME) | Mink | TME prion | $MkPrP^{Sc}$ |
| 90.001.0.01.003. | Chronic wasting disease (CWD) | Elk, White-tailed deer, Mule Deer and Red Deer | CWD prion | $MDePrP^{Sc}$ |
| 90.001.0.01.004. | Bovine spongiform encephalopathy (BSE) commonly known as "Mad Cow Disease" | Cattle | BSE prion | $BovPrP^{Sc}$ |
| 90.001.0.01.005. | Feline spongiform encephalopathy (FSE) | Cats | FSE prion | $FePrP^{Sc}$ |
| 90.001.0.01.006. | Exotic ungulate encephalopathy (EUE) | Nyala and greater kudu | EUE prion | $NyaPrP^{Sc}$ |
| | **Human diseases** | | | |
| 90.001.0.01.007. | Kuru | Humans | Kuru prion | $HuPrP^{Sc}$ |
| 90.001.0.01.008. | Creutzfeldt-Jakob disease (CJD) | | CJD prion | |
| | (New) Variant Creutzfeldt-Jakob disease (vCJD, nvCJD) | | vCJD prion | |
| 90.001.0.01.009. | Gerstmann-Sträussler-Scheinker syndrome (GSS) | | GSS prion | |

| | | |
|---|---|---|
| 90.001.0.01.010. | Fatal familial insomnia (FFI) | FFI prion |

## *Features of TSE*

The degenerative tissue damage caused by human prion diseases (CJD, GSS, and kuru) is characterised by four features: spongiform change, neuronal loss, astrocytosis and amyloid plaque formation. These features are shared with prion diseases in animals, and the recognition of these similarities prompted the first attempts to transmit a human prion disease (kuru) to a primate in 1966, followed by CJD in 1968 and GSS in 1981. These neuropathological features have formed the basis of the histological diagnosis of human prion diseases for many years, although it was recognized that these changes are enormously variable both from case to case and within the central nervous system in individual cases.

The clinical signs in humans vary, but commonly include personality changes, psychiatric problems such as depression, lack of coordination, and/or an unsteady gait (ataxia). Patients also may experience involuntary jerking movements called myoclonus, unusual sensations, insomnia, confusion, or memory problems. In the later stages of the disease, patients have severe mental impairment (dementia) and lose the ability to move or speak.

Early neuropathological reports on human prion diseases suffered from a confusion of nomenclature, in which the significance of the diagnostic feature of spongiform change was occasionally overlooked. The subsequent demonstration that human prion diseases were transmissible reinforced the importance of spongiform change as a diagnostic feature, reflected in the use of the term "spongiform encephalopathy" for this group of disorders.

Prions appear to be most infectious when in direct contact with affected tissues. For example, Creutzfeldt-Jakob disease has been transmitted to patients taking injections of growth hormone harvested from human pituitary glands, from cadaver dura allografts and from instruments used for brain surgery (Brown, 2000) (prions can survive the "autoclave" sterilization process used for most surgical instruments). It is also believed that dietary consumption of affected animals can cause prions to accumulate slowly, especially when cannibalism or similar practices allow the proteins to accumulate over more than one generation. An example is kuru, which reached epidemic proportions in the mid 20th century in the Fore people of Papua New Guinea, who used to consume their dead as a funerary ritual. Laws in developed countries now proscribe the use of rendered ruminant proteins in ruminant feed as a precaution against the spread of prion infection in cattle and other ruminants.

Note that not all encephalopathies are caused by prions, as in the cases of PML (caused by the JC virus), CADASIL (caused by abnormal NOTCH3 protein activity), and Krabbe disease (caused by a deficiency of the enzyme galactosylceramidase). PSL -- which is a spongiform encephalopathy—is also probably not caused by a prion, although the

adulterant which causes it among heroin smokers has not yet been identified. This, combined with the highly variable nature of prion disease pathology, is why a prion disease cannot be diagnosed based solely on a patient's symptoms.

## *Genetics*

Mutations in the PRNP gene cause prion disease. Familial forms of prion disease are caused by inherited mutations in the PRNP gene. Only a small percentage of all cases of prion disease run in families, however. Most cases of prion disease are sporadic, which means they occur in people without any known risk factors or gene mutations. Rarely, prion diseases also can be transmitted by exposure to prion-contaminated tissues or other biological materials obtained from individuals with prion disease.

The PRNP gene provides the instructions to make a protein called the prion protein (PrP). Normally, this protein may be involved in transporting copper into cells. It may also be involved in protecting brain cells and helping them communicate. 24 Point-Mutations in this gene cause cells to produce an abnormal form of the prion protein, known as $PrP^{Sc}$. This abnormal protein builds up in the brain and destroys nerve cells, resulting in the signs and symptoms of prion disease.

Familial forms of prion disease are inherited in an autosomal dominant pattern, which means one copy of the altered gene in each cell is sufficient to cause the disorder. In most cases, an affected person inherits the altered gene from one affected parent.

In some people, familial forms of prion disease are caused by a new mutation in the PRNP gene. Although such people most likely do not have an affected parent, they can pass the genetic change to their children.

## *Competing hypotheses*

### Protein-Only hypothesis

Protein could be the infectious agent, inducing its own replication by causing conformational change of normal cellular $PrP^{C}$ into $PrP^{Sc}$. Evidence for this theory:

- infectivity titre correlates with $PrP^{Sc}$ levels. However, this is disputed.
- $PrP^{Sc}$ is an isomer of $PrP^{C}$
- Denaturing PrP removes infectivity
- PrP-null mice cannot be infected

### Multi-component hypothesis

While not containing a nucleic acid genome, prions may be composed of more than just a protein. Purified $PrP^{C}$ appears unable to convert to the infectious $PrP^{Sc}$ form, unless other components are added, such as RNA and lipids. These other components, termed

cofactors, may form part of the infectious prion, or they may serve as catalysts for the replication of a protein-only prion.

### Viral hypothesis

This hypothesis postulates that an infectious viral agent is the cause of the disease. Evidence for this hypothesis is as follows:

- Incubation time is comparable to a lentivirus
- Strain variation of different isolates of $PrP^{Sc}$
- An increasing titre of $PrP^{Sc}$ as the disease progresses suggests a replicating agent.

This hypothesis is largely discredited, as no infectious, non-human nucleic acid has ever been isolated from the disease. It is largely based on the fact that infectious agents have previously been viral in origin, preferring this as more plausible than the infectious protein hypothesis.

## *Epidemiology*

These spontaneous disorders in humans are very rare, affecting only about one person per million worldwide each year. However, transmissible TSEs can reach epidemic proportions, as was seen in the UK BSE outbreak of the 80s and 90s. It is very hard to map the spread of the disease due to the difficulty of identifying individual strains of the prions. This means that if animals start to show the disease after an outbreak on a nearby farm, you cannot show that it is the same strain affecting both, suggesting transmission, or that the second outbreak came from a completely different source.

## *Possible cure or vaccine*

Recent research from the University of Toronto and Caprion Pharmaceuticals have discovered one possible avenue which might lead to quicker diagnosis, a vaccine or possibly even treatment for prion diseases. The abnormally folded proteins which cause the disease have been found to expose a side chain of amino acids which the properly folded protein does not expose. Antibodies specifically coded to this side chain amino acid sequence have been found to stimulate an immune response to the abnormal prions and leave the normal proteins intact.

Another idea involves using custom peptide sequences. Since some research suggests prions aggregate by forming beta barrel structures, work done *in vitro* has shown that peptides made up of beta barrel-incompatible amino acids can help break up accumulations of prion. Yet a third idea concerns genetic therapy, whereby the gene for encoding protease-resistant protein is considered to be an error in several species, and therefore something to be inhibited.

Chapter 6

# Chronic Wasting Disease and Scrapie

## Chronic wasting disease

**Chronic wasting disease** (**CWD**) is a transmissible spongiform encephalopathy (TSE) of mule deer, whitetailed deer, elk (wapiti), and moose. TSEs are caused by unusual infectious agents known as prions. To date, CWD has been found mainly in cervids (members of the deer family). First recognized as a clinical "wasting" syndrome in 1967 in mule deer in a wildlife research facility in northern Colorado, USA, it was identified as a TSE in 1978 and has spread to a dozen states and two Canadian provinces. CWD is typified by chronic weight loss leading to death. There is no known relationship between CWD and any other TSE of animals or people.

Although there have been reports in the popular press of humans being affected by CWD, a study by the CDC suggests that "[m]ore epidemiologic and laboratory studies are needed to monitor the possibility of such transmissions." The epidemiological study further concludes that, "[a]s a precaution, hunters should avoid eating deer and elk tissues known to harbor the CWD agent (e.g., brain, spinal cord, eyes, spleen, tonsils, lymph nodes) from areas where CWD has been identified."

### *Clinical signs*

Most cases of CWD occur in adult animals. The disease is progressive and always fatal. The most obvious and consistent clinical sign of CWD is weight loss over time. Behavioral changes also occur in the majority of cases, including decreased interactions with other animals, listlessness, lowering of the head, blank facial expression, repetitive walking in set patterns, and a smell like meat starting to rot. In elk, behavioral changes may also include hyperexcitability and nervousness. Affected animals continue to eat grain but may show decreased interest in hay. Excessive salivation and grinding of the teeth also are observed. Most deer show increased drinking and urination.

## Causative agent

The agent responsible for CWD (and other TSEs, such as scrapie and bovine spongiform encephalopathy) is a prion, an abnormal form of a normal protein, known as prion protein (PrP), most commonly found in the central nervous system (CNS), and is capable of spreading to the peripheral nervous system (PNS), thus infecting meat, or muscle, of deer and elk. The abnormal prion protein infects the host animal by promoting conversion of normal cellular prion protein ($PrP^{C}$) to the abnormal prion form ($PrP^{CWD}$). The build-up of $PrP^{CWD}$ in the brain is associated with widespread neurodegeneration.

## Diagnosis

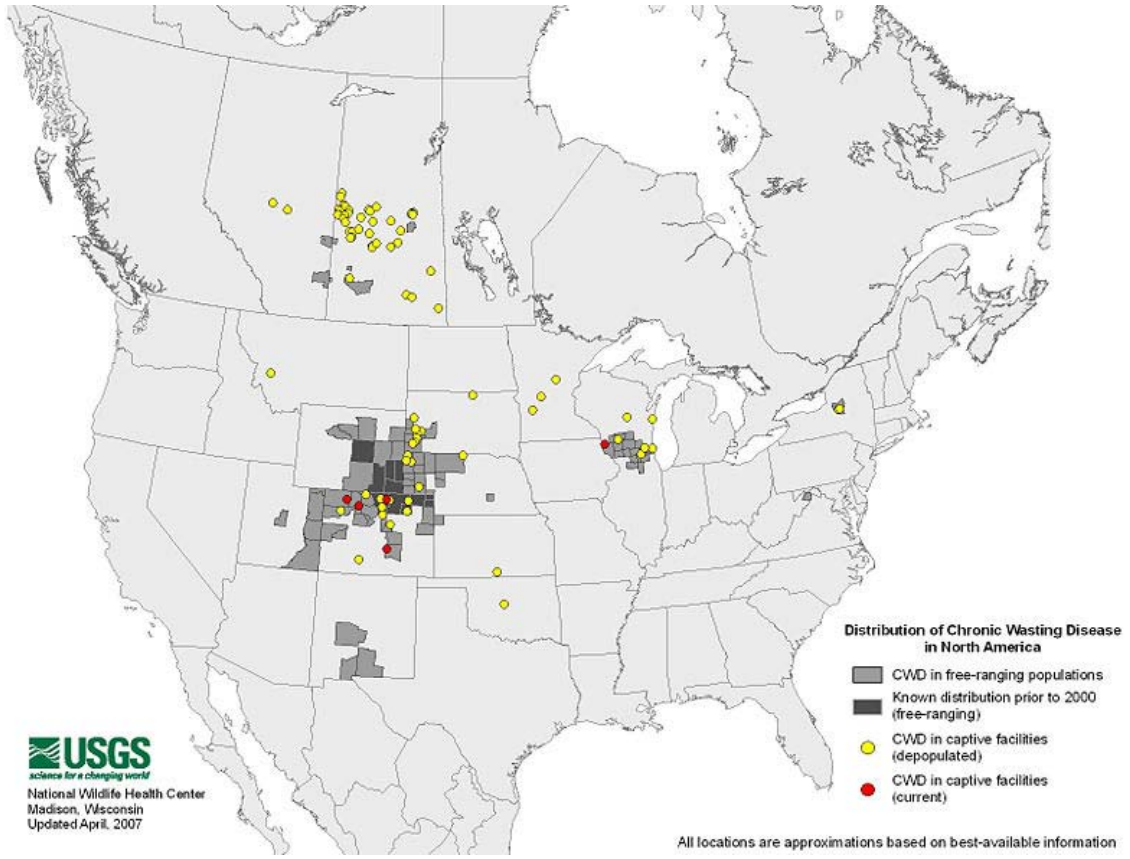
Research is being conducted to develop live-animal diagnostic tests for CWD. Currently, definitive diagnosis is based on postmortem examination (necropsy) and testing. Gross lesions seen at necropsy reflect the clinical signs of CWD, primarily emaciation. Aspiration pneumonia, which may be the actual cause of death, also is a common finding in animals affected with CWD. On microscopic examination, lesions of CWD in the central nervous system resemble those of other TSEs. In addition, scientists use a technique called immunohistochemistry to test brain tissue for the presence of the abnormal prion protein to diagnose CWD.

## Epidemiology

The origin and mode of transmission of the prions causing CWD is unknown, but recent research indicates that prions can be excreted by deer and elk, and is transmitted by eating grass growing in contaminated soil. Animals born in captivity and those born in the wild have been affected with the disease. Based on epidemiology, transmission of CWD is thought to be lateral (from animal to animal). Maternal transmission may occur, although it appears to be relatively unimportant in maintaining epidemics. Research has recently shown that an infected deer's saliva is able to spread the CWD prions.

In the mid-1980s, CWD was detected in free-ranging deer and elk in contiguous portions of northeastern Colorado and southeastern Wyoming. Soon after diagnosis of the disease as a TSE, Colorado and Wyoming wildlife management agencies stopped the movement of deer and elk from their research facilities; wild cervids have not been translocated from the endemic area. In May 2001, CWD was also found in free-ranging deer in the southwestern corner of Nebraska (adjacent to Colorado and Wyoming) and later in additional areas in western Nebraska. The limited area of northern Colorado, southern Wyoming, and western Nebraska in which free-ranging deer, moose, and/or elk positive for CWD have been found is referred to as the endemic area. The area in 2006 has expanded to six states including parts of eastern Utah, southwestern South Dakota, and northeastern Kansas. There are also non-contiguous (to the endemic area) areas in central Utah and central Nebraska. The limits of the affected areas are not well defined since the disease is at a low incidence and the amount of sampling may not be adequate to detect it. In 2002, CWD was detected in wild deer in south-central Wisconsin and northern Illinois and in an isolated area of southern New Mexico. In 2005, it was found in wild White-

tailed deer in New York and in Hampshire County, West Virginia. In 2008, the first confirmed case of CWD in Michigan was discovered in an infected deer on an enclosed deer breeding facility. It is also found in the Canadian provinces of Alberta and Saskatchewan.




Chronic wasting disease in North America

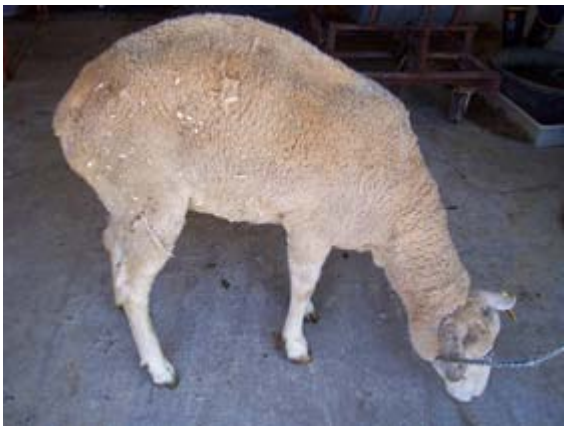
In February of 2011, the Maryland Department of Natural Resources reported the first confirmed case of the disease in that state. The affected animal was a white-tailed deer killed by a hunter.

CWD has also been diagnosed in farmed elk and deer herds in a number of states and in two Canadian provinces. The first positive farmed elk herd in the United States was detected in 1997 in South Dakota. Since then, additional positive elk herds and farmed White-tailed deer herds have been found in South Dakota (7), Nebraska (4), Colorado (10), Oklahoma (1), Kansas (1), Minnesota (3), Montana (1), Wisconsin (6) and New York (2). As of fall of 2006, four positive elk herds in Colorado and a positive White-tailed deer herd in Wisconsin remain under State quarantine. All of the other herds have been depopulated or have been slaughtered and tested, and the quarantine has been lifted from one herd that underwent rigorous surveillance with no further evidence of disease. CWD also has been found in farmed elk in the Canadian provinces of Saskatchewan and

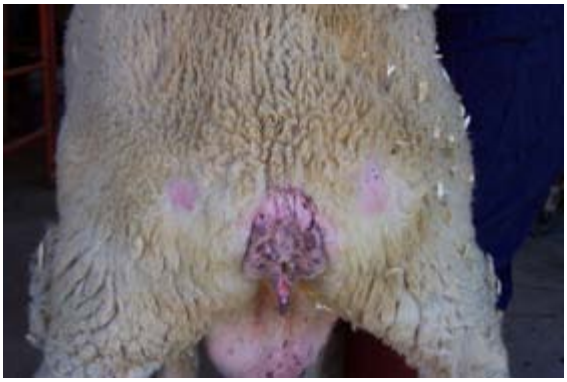
Alberta. A retrospective study also showed that Mule deer exported from Denver to the Toronto Zoo in the 1980s were affected.

Species that have been affected with CWD include elk, mule deer, white-tailed deer, black-tailed deer, and moose. Other ruminant species, including wild ruminants and domestic cattle, sheep, and goats, have been housed in wildlife facilities in direct or indirect contact with CWD-affected deer and elk with no evidence of disease transmission. There is ongoing research to further explore the possibility of transmission of CWD to other species.

# Scrapie



Ewe with scrapie with weight loss and hunched appearance



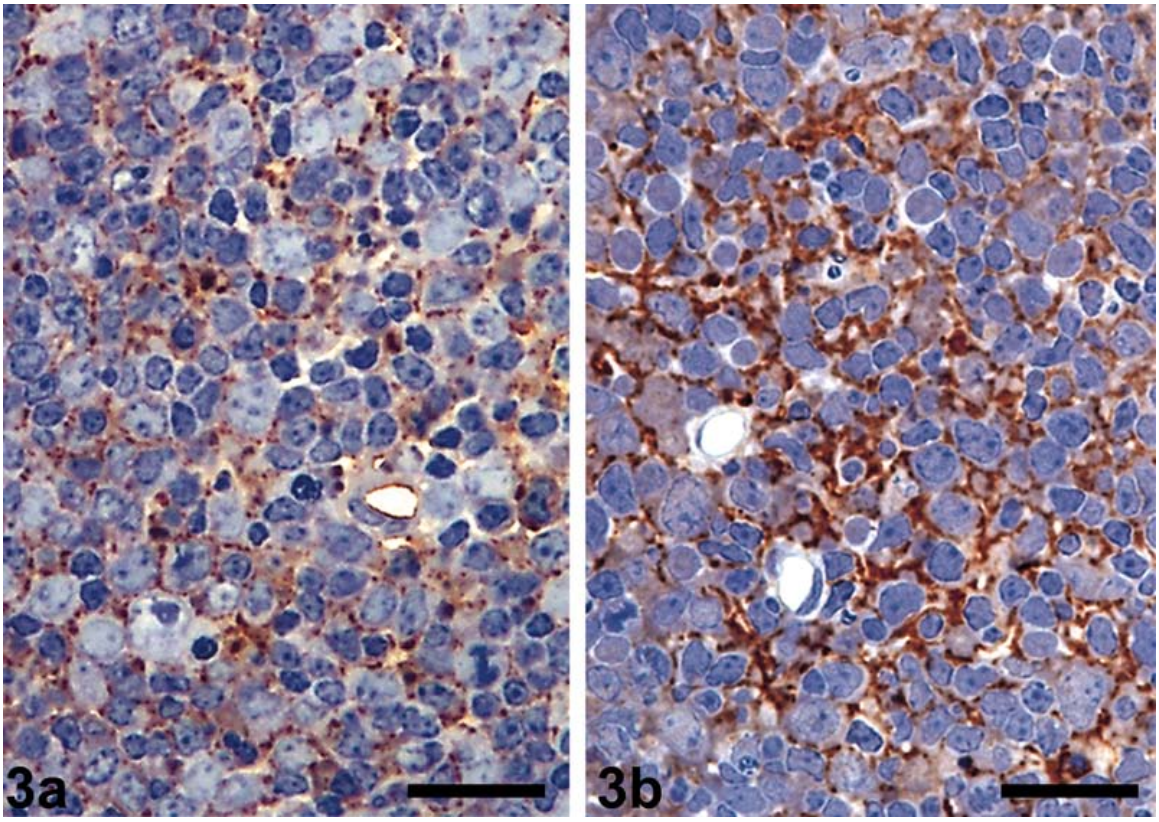
Same ewe as above with bare patches on rear end from scraping

**Scrapie** is a fatal, degenerative disease that affects the nervous systems of sheep, goats and occasionally even wasps. It is one of several transmissible spongiform encephalopathies (TSEs), which are related to bovine spongiform encephalopathy (BSE or "mad cow disease") and chronic wasting disease of deer. Like other spongiform encephalopathies, scrapie is caused by a prion. Scrapie has been known since the 18th century (1732) and does not appear to be transmissible to humans.

The name scrapie is derived from one of the clinical signs of the condition, wherein affected animals will compulsively scrape off their fleece against rocks, trees or fences. The disease apparently causes an itching sensation in the animals. Other clinical signs include excessive lip-smacking, altered gaits, and convulsive collapse.

Scrapie is infectious and transmissible among similar animals, and so one of the most common ways to contain scrapie (since it is incurable) is to quarantine and destroy those affected. However, scrapie tends to persist in flocks and can also arise apparently spontaneously in flocks that have not previously had cases of the disease. The mechanism of transmission between animals and other aspects of the biology of the disease are only poorly understood and these are active areas of research. Recent studies suggest that prions may be spread through urine and persist in the environment for decades.

## *Uptake of prions*




Lymph nodes from (a) healthy and (b) infected sheep. Colouring with antibodies shows clear sign of scrapie prions in the intracellular tissue of the infected sheep.

The protein enters through the intestines or through cuts in the skin. The prions cause normal proteins of the sheep to fold into the wrong shape. These proteins are gradually accumulated in the body, especially in nerve cells which subsequently die. When the prions are absorbed through the intestines, they first appear in the lymph nodes, especially in Peyer's patches at the small intestine.

An experiment has shown that lambs risk being infected through milk from infected ewes. But the lambs in the experiment also infected each other, making it difficult to assess the risk of infection. The experiment did not continue long enough to show that the lambs developed symptoms, merely that the prion was present in the body.

## *Preventive action*

A test is now available which is performed by sampling a small amount of lymphatic tissue from the third eyelid.

In the United Kingdom, the government has put in place a National Scrapie Plan, which encourages breeding from sheep that are genetically more resistant to scrapie. It is intended that this will eventually reduce the incidence of the disease in the UK sheep population. Scrapie occurs in Europe and North America, but to date Australia and New Zealand (both major sheep-producing countries) are scrapie-free.

Breeds such as cheviot sheep and suffolk are more susceptible to scrapie than other breeds. Specifically, this is determined by the genes coding for the naturally occurring prion proteins. The most resistant sheep have a double set of "ARR" alleles, while sheep with the "VRQ" allele are the most susceptible. A simple blood test reveals the allele of the sheep and many countries are actively breeding away the VRQ allele.

Out of fear of BSE, many European countries banned some traditional sheep or goat products made without removing the spinal cord such as smalahove and smokie.

Chapter 7

# Kuru (Disease) and Fatal Familial Insomnia

# Kuru

| Kuru | |
|---|---|
| **ICD-10** | A81.8 |
| **ICD-9** | 046.0 |
| **OMIM** | 245300 |
| **DiseasesDB** | 31861 |
| **MedlinePlus** | 001379 |
| **eMedicine** | med/1248 |
| **MeSH** | D007729 |

**Kuru** is an incurable degenerative neurological disorder (brain disease) that is a type of transmissible spongiform encephalopathy, caused by a prion found in humans. The term "kuru" derives from the Fore word "kuria/guria", 'to shake'., a reference to the body tremors that are a classic symptom of the disease; it is also known among the Fore as the *laughing sickness* due to the pathologic bursts of laughter people would display when afflicted with the disease. It is now widely accepted that Kuru was transmitted among members of the Fore tribe of Papua New Guinea via cannibalism.

## *History*

Kuru was first noted in the Fore tribe of the Eastern Highlands Province of Papua New Guinea as Australian administrators explored the area in 1953–1959. Kuru (Keru) was reported by W. T. Brown in Kainantu Patrol Report No 8 of 1953/54 (13 January 1954 - 20 February 1954.) .. "The first sign of impending death is a general debility which is followed by general weakness and inability to stand. The victim retires to her house. She is able to take a little nourishment but suffers from violent shivering. The next stage is that the victim lies down in the house and cannot take nourishment and death eventually

ensues." The same reports described the cannibalism practised by the Fore people. It was in the late 1950s that the full extent of the disease was realized, after it had reached large infection rates in the South Fore of the Okapa Subdistrict, though the agent was unknown.

The disease was researched by Daniel Carleton Gajdusek as part of an international collaboration with Australian doctor (now Professor) Michael Alpers. In the mid-1960s Alpers collected post-mortem brain tissue samples from an 11-year-old Fore girl, Kigea, who had died of kuru. He took these samples to Gajdusek in the USA, who then injected two chimpanzees with the infected material. Within two years, one of the chimps, Daisy, had developed kuru, demonstrating that the unknown disease factor was transmitted through infected biomaterial and that it was capable of crossing the species barrier to other primates.

In 1976 Gajdusek, along with Baruch S. Blumberg, was awarded the Nobel Prize in Physiology or Medicine for showing that kuru was transmissible to chimpanzees. This was the first time that this group of encephalopathies had been demonstrated to be infectious and therefore a major step forwards in their investigation. As kuru is the only epidemic of human prion disease in known human history, it has provided important insights into the variant CJD.

## *Causes*

Kuru is believed to be caused by prions and is related to Creutzfeldt-Jakob disease. It is best known for the epidemic that occurred in Papua New Guinea in the middle of the twentieth century, and earlier. Although it is considered a transmissible prion disease, there is some evidence that the origin of the outbreak was due to consumption of an individual (cannibalism) with sporadic CJD, thus implying a common pathophysiology.

## *Presentation*

Kuru causes physiological as well as neurological effects that ultimately lead to death. It was endemic among the Fore tribe of Papua New Guinea and was confined to the Fore population and those nearby populations with whom they intermarried. It is characterized by truncal ataxia, preceded by headaches, joint pains and shaking of the limbs. Trembling is present in almost all patients with transmissible spongiform encephalopathy, Kuru is also known as "shiver." The preclinical or asymptomatic phase, also called the incubation period, lasts between possibly 5 to 20 years following initial exposure. The clinical stage lasts an average of 12 months.

## *Transmission*

Beginning in 1961, Australian doctor (now Professor) Michael Alpers conducted extensive field studies among the Fore, which were supported by the work of anthropologist Shirley Lindenbaum. Their historical research with the Fore suggests that the epidemic may have originated around 1900 from a single individual who lived on the

edge of Fore territory, who is thought to have spontaneously developed some form of Creutzfeldt-Jakob Disease (CJD). Alpers and Lindenbaum's research conclusively demonstrated that kuru spread easily and rapidly in the Fore people due to their endocannibalistic funeral practices, in which relatives consumed the bodies of the deceased to return the "life force" of the deceased to the hamlet, a Fore societal subunit. The dysmorphism evident in the infection rates — kuru was 8–9 times more prevalent in women and children than in men at its peak — is because while the men of the village took the choice cuts, the women and children would eat the rest of the body including the brain, where the prion particles were particularly concentrated. There is also the strong possibility that it was passed on to women and children more easily because they took on the task of cleaning relatives after death and may have had open sores and cuts on their hands. Although ingestion itself of the prion particles can lead to the disease, there was a high degree of transmission if the prion particles could reach the subcutaneous tissue. With elimination of cannibalism because of Australian colonial law enforcement and the local Christian missionaries' efforts, Alpers' research showed that Kuru was already declining among the Fore by the mid-1960s, although cases continued to appear for several decades more, and the last sufferer died in 2005. However, the mean incubation period of the disease is 14 years and cases were reported with latencies of 40 years or more for those who were most genetically resilient.

### *Immunity*

Simon Mead of University College London, and others, showed in their genetic and clinical assessment that people who survived the epidemic in Papua New Guinea were carriers of a prion resistant factor. Mead's group has shown the source of immunity to be the inheritance of a genetic variant of prion protein G127V. This work remains breaking news as of November 22, 2009, and further implications of the discovery including evidence for rapid natural selection of populations are being discussed.

# Fatal familial insomnia

## Fatal familial insomnia

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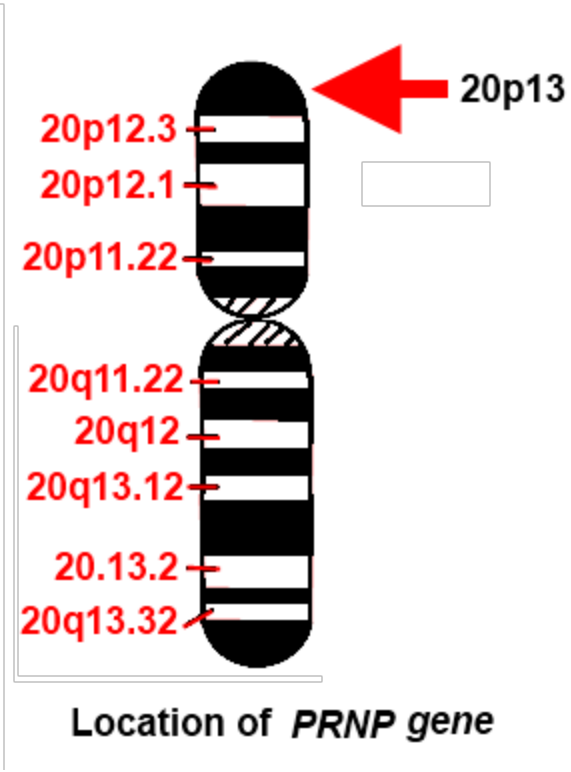
|---|---|
| **ICD-10** | A81.9 |
| **ICD-9** | 046.72 |
| **OMIM** | 600072 |
| **DiseasesDB** | 32177 |
| **MeSH** | D034062 |

**Fatal familial insomnia** (FFI) is a very rare autosomal dominant inherited prion disease of the brain. It is almost always caused by a mutation to the protein $PrP^C$, but it can also develop spontaneously in patients without the inherited mutation in a variant called

**sporadic fatal insomnia** (SFI). The mutated protein, called $PrP^{Sc}$, has been found in just 40 families worldwide, affecting about 100 people; if only one parent has the gene, the offspring have a 50% chance of inheriting it and developing the disease. The disease's genesis and the patient's progression into complete sleeplessness is untreatable and ultimately fatal.

## *The prion*




Chromosome 20

Gene PRNP that provides instructions for making the prion protein $PrP^{C}$ is located on the short (p) arm of chromosome 20 at position 13. Both FFI patients and those with Creutzfeldt-Jakob disease carry a mutation at codon 178 of the prion protein gene. FFI is invariably linked to the presence of the methionine codon at position 129 of the mutant allele, whereas CJD is linked to the presence of the valine codon at that position.

## *Presentation*

The age of onset is variable, ranging from 35 to 60, with an average of 50. However the disease tends to prominently occur in later years, primarily following childbirth. The disease can be detected prior to onset by genetic testing. Death usually occurs between 7 and 36 months from onset. The presentation of the disease varies considerably from person to person, even among patients from within the same family.

The disease has four stages, taking 7 to 18 months to run its course:

1. The patient suffers increasing insomnia, resulting in panic attacks, paranoia, and phobias. This stage lasts for about four months.
2. Hallucinations and panic attacks become noticeable, continuing for about five months.
3. Complete inability to sleep is followed by rapid loss of weight. This lasts for about three months.
4. Dementia, during which the patient becomes unresponsive or mute over the course of six months. This is the final progression of the disease, resulting in death.

Other symptoms include profuse sweating, pinprick pupils, the sudden entrance into menopause for women and impotence for men, neck stiffness, and elevation of blood pressure and heart rate. Constipation is common as well.

## *Treatment*

There is no cure or treatment for FFI. Gene therapy is so far unsuccessful. While it is not currently possible to reverse the underlying illness, there is some evidence that treatments that focus upon the symptoms can improve quality of life.

Several cases have proven that sleeping pills and barbiturates do not help, they make FFI worse and actually speed up the disease.

In the late 2000s, a mouse model was made for FFI. These mice express a humanized version of the PrP protein that also contains the D178N FFI mutation. These mice appear to have progressively fewer and shorter periods of uninterrupted sleep, damage in the thalamus, and early deaths, similar to people with FFI.

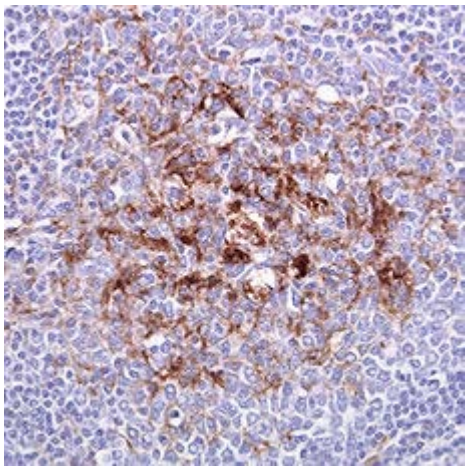
## *Related conditions*

There are other diseases involving the mammalian prion. Some are transmissible (TSEs) such as kuru, bovine spongiform encephalopathy (BSE, also known as "mad cow disease") in cows, and chronic wasting disease in American deer and American elk in some areas of the United States and Canada, as well as Creutzfeldt-Jakob disease (CJD). Until recently these were not considered to be transmissible except by direct contact with infected tissue, such as from eating infected tissue, transfusion or transplantation; now it is believed prion diseases are subject to airborne transmission.

## Chapter 8

# Creutzfeldt–Jakob Disease

| Creutzfeldt–Jakob disease |
|---|



Tonsil biopsy in variant CJD. Prion Protein immunostaining.

| | |
|---|---|
| **ICD-10** | A81.0, F02.1 |
| **ICD-9** | 046.1 |
| **OMIM** | 123400 |
| **DiseasesDB** | 3166 |
| **eMedicine** | neuro/725 |
| **MeSH** | D007562 |

**Creutzfeldt–Jakob disease** or **CJD** is a degenerative neurological disorder (brain disease) that is incurable and invariably fatal. The disease is at times called a human form of **Mad Cow disease** given the fact that Bovine spongiform encephalopathy is the cause of variant Creutzfeldt-Jakob disease in humans.

It is the most common among the types of transmissible spongiform encephalopathy found in humans. This means that the brain develops holes and takes on a sponge-like texture. This is caused by a type of infectious protein called a prion, which destroys the brain while reproducing, similar to a virus. It is usually transmitted by eating contaminated beef.

The disease is always fatal. There is no cure, and all treatments are experimental.

## Classification

Types include:

- sporadic (sCJD)
- variant (vCJD) This type is more likely to be acquired. It can be iatrogenic. It was first identified in 1996.
- familial (fCJD)

## Signs and symptoms

The first symptom of CJD is rapidly progressive dementia, leading to memory loss, personality changes and hallucinations. This is accompanied by physical problems such as speech impairment, jerky movements (myoclonus), balance and coordination dysfunction (ataxia), changes in gait, rigid posture, and seizures. The duration of the disease varies greatly, but sporadic (non-inherited) CJD can be fatal within months or even weeks (Johnson, 1998). In some people, the symptoms can continue for years. In most patients, these symptoms are followed by involuntary movements and the appearance of an atypical diagnostic electroencephalogram tracing.

The symptoms of CJD are caused by the progressive death of the brain's nerve cells, which is associated with the build-up of abnormal prion proteins. When brain tissue from a CJD patient is examined under a microscope, many tiny holes can be seen where whole areas of nerve cells have died. The word "spongiform" in "transmissible spongiform encephalopathies" refers to the sponge-like appearance of the brain tissue.

## Cause

Transmissible spongiform encephalopathy diseases are caused by prions. The diseases are thus sometimes called prion diseases. Other prion diseases include Gerstmann–Sträussler–Scheinker syndrome (GSS), fatal familial insomnia (FFI) and kuru in humans, as well as bovine spongiform encephalopathy (BSE, commonly known as mad cow disease) in cattle, chronic wasting disease (CWD) in elk and deer, and scrapie in sheep. Alpers' syndrome in infants is also thought to be a transmissible spongiform encephalopathy caused by a prion.

The prion that is believed to cause Creutzfeldt–Jakob exhibits at least two stable conformations. One, the native state, is water-soluble and present in healthy cells. As of 2007, its biological function is presumably in transmembrane transport or signaling. The other conformational state is very poorly water-soluble and readily forms protein aggregates.

People can also acquire CJD genetically through a mutation of the gene that codes for the prion protein (PRNP). This occurs in only 5–10% of all CJD cases.

The CJD prion is dangerous because it promotes refolding of native proteins into the diseased state,. The number of misfolded protein molecules will increase exponentially and the process leads to a large quantity of insoluble prions in affected cells. This mass of misfolded proteins disrupts cell function and causes cell death. Mutations in the gene for the prion protein can cause a misfolding of the dominantly alpha helical regions into beta pleated sheets. This change in conformation disables the ability of the protein to undergo digestion. Once the prion is transmitted, the defective proteins invade the brain and are produced in a self-sustaining feedback loop, causing exponential spread of the prion, leading to death within a few months, although a few patients have lived as long as two years.

Stanley B. Prusiner of the University of California, San Francisco (UCSF) was awarded the Nobel Prize in physiology or medicine in 1997 for his discovery of prions. For more than a decade, Yale University neuropathologist Laura Manuelidis has been challenging this explanation for the disease. In January 2007, she and her colleagues published an article in the *Proceedings of the National Academy of Science* and reported that they have found a virus-like particle (but without finding nucleic acids so far) in less than 10% of the cells a scrapie-infected cell line and in a mouse cell line infected by a human CJD agent.

## Transmission

The defective protein can be transmitted by contaminated harvested human growth hormone (HGH) products, Immunoglobulins (IVIG), corneal grafts, dural grafts or electrode implants (acquired or iatrogenic form: iCJD); it can be inherited (hereditary or familial form: fCJD); or it may appear for the first time in the patient (sporadic form: sCJD). In the hereditary form, a mutation occurs in the gene for PrP, PRNP. Ten to fifteen percent of CJD cases are inherited. (CDC)

The disease has also been shown to result from usage of HGH drawn from the pituitary glands of cadavers who died from Creutzfeldt–Jakob Disease, though the known incidence of this cause is (as of April 2004) quite small. The risk of infection through cadaveric HGH usage in the US only ceased when the medication was withdrawn in 1985.

It is thought that humans can contract the disease by consuming material from animals infected with the bovine form of the disease. The only suspected cases to arise thus far

have been vCJD, although there are fears—based on animal studies—that consuming beef or beef products containing prion particles can also cause the development of classic CJD. When BSE material infects humans the resulting disease is known as (new) variant CJD (nvCJD).

Cannibalism has also been implicated as a transmission mechanism for abnormal prions, causing the disease known as kuru, found primarily among women and children of the Fore tribe in Papua New Guinea. While the men of the tribe ate the body of the deceased and rarely contracted the disease, the women and children, who ate the less desirable body parts, were 8 times more likely to contract the disease from infected tissue.

Prions, the infectious agent of CJD, may not be inactivated by means of routine surgical instrument sterilization procedures. The World Health Organization and the US Centers for Disease Control and Prevention recommend that instrumentation used in such cases be immediately destroyed after use; secondary to destruction, it is recommended that heat and chemical decontamination be used in combination to process instruments that come in contact with high-infectivity tissues. No cases of iatrogenic transmission of CJD have been reported subsequent to the adoption of current sterilization procedures, or since 1976. Copper–hydrogen peroxide has been suggested as an alternative to the current recommendation of sodium hydroxide or sodium hypochlorite. Thermal depolymerization also destroys prions in infected organic and inorganic matter, since the process dissolves protein at the molecular level.

## Blood donor restrictions

In 2004 a new report published in the *Lancet* medical journal showed that vCJD can be transmitted by blood transfusions. The finding alarmed healthcare officials because a large epidemic of the disease might arise in the near future. There is no test to determine if a blood donor is infected while in the latent phase of vCJD. In reaction to this report, the UK government banned anyone who had received a blood transfusion since January 1980 from donating blood. From 1999 there has been a ban in the UK for using UK blood to manufacture fractional products such as albumin.

On May 28, 2002, the United States Food and Drug Administration instituted a policy that excludes from donation anyone who spent at least six months in certain European countries, (or three months in the United Kingdom), from 1980 to 1996. Given the large number of U.S. military personnel and their dependents residing in Europe, it was expected that over 7% of donors would be deferred due to the policy. Later changes to this policy have relaxed the restriction to a cumulative total of five years or more of civilian travel in European countries (six months or more if military). The three-month restriction on travel to the UK, however, has not been changed.

The American Red Cross' policy is as follows: During the period January 1, 1980, to December 31, 1996, spending a total time of three months or more in the Channel Islands, England, the Falkland Islands, the Isle of Man, Gibraltar, Northern Ireland, Scotland, and Wales precludes individuals from donating. Moreover, spending a total

time of five years or more after January 1, 1980 (to present), in the above-mentioned countries and/or any country in Europe (except the former USSR), also precludes donation. People with a biologic relative who has been diagnosed with CJD or vCJD are unable to donate. Biologic relative in this setting means mother, father, sibling, grandparent, aunt, uncle or children. (For complete listing, please go to Redcross.org)

A similar policy applies to potential donors to the Australian Red Cross' Blood Service, precluding people who have spent a cumulative time of six months or more in the United Kingdom between 1980 and 1996.

The Singapore Red Cross precludes potential donors who have spent a cumulative time of three months or more in the United Kingdom between 1980 and 1996.

In New Zealand, anyone who has lived in the UK, France or the Republic of Ireland for a total of six months or more between 1980 and 1996 is prohibited from donating blood.

Similar regulations are in place in Germany, where anyone who has spent six months or more living in the UK between January 1980 and December 1996 is permanently barred from donating blood.

As of 1999, Health Canada announced a policy to defer individuals from donating blood if they have lived within the United Kingdom for one month or more from January 1, 1980, to December 31, 1996. In 2000, the same policy was applied to people who have resided in France, for at least three months from January 1980 to December 1996. Canada will not accept blood from a person who has spent more than six months in a Western European country since January 1, 1980.

The Association of Blood Donors of Denmark precludes potential donors who have spent a cumulative time of at least twelve months in the United Kingdom between 1 January 1980 and 31 December 1996.

The Swiss Blutspendedienst SRK precludes potential donors who have spent a cumulative time of at least six months in the United Kingdom between 1 January 1980 and 31 December 1996.

In Poland, anyone who cumulatively spent six months or longer between 1 January 1980 and 31 December 1996 in the UK, Ireland or France is permanently barred from donating.

In the Czech Republic, anyone who spent more than six months in the UK or France between the years 1980 and 1996 or received transfusion in the UK after the year 1980 is not allowed to donate blood.

### Sperm donor restrictions

In the U.S., the FDA has banned import of any donor sperm, motivated by a risk of Creutzfeldt–Jakob disease, inhibiting the once popular import of, for example, Scandinavian sperm. The risk, however, is not known, since artificial insemination has not been studied as a route of transmission. It is also not known whether prions cross the blood-testis barrier.

## *Diagnosis*

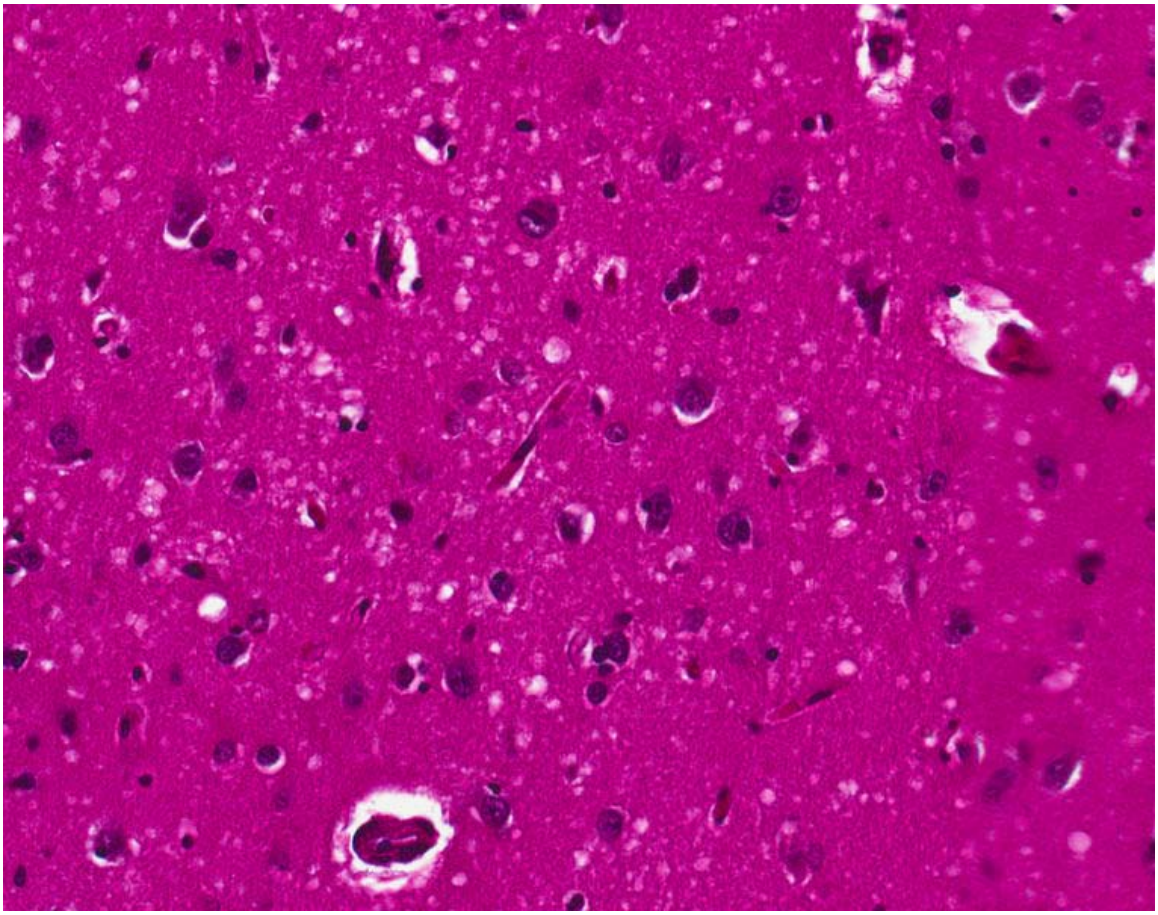
The diagnosis of CJD is suspected when there are typical clinical symptoms and signs such as rapidly progressing dementia with myoclonus. Further investigation can then be performed to support the diagnosis including

- Electroencephalography — often has characteristic triphasic spikes
- Cerebrospinal fluid analysis for 14-3-3 protein
- MRI of the brain — often shows high signal intensity in the caudate nucleus and putamen bilaterally on T2-weighted images.
- Research in 2010 and 2011 identified a possible blood test for CJD. The test attempts to identify the prion responsible for the disease. However, it was unable to detect the prions in those in early stages of the disease.

Diffusion Weighted Imaging (DWI) images are the most sensitive. In about 24% of cases DWI shows only cortical hyperintensity; in 68%, cortical and subcortical abnormalities; and in 5%, only subcortical anomalies. The involvement of the thalamus can be found in sCJD, is even stronger and constant in vCJD.

Clinical testing for CJD has always been an issue. Diagnosis has mostly been based on clinical and physical examination of symptoms. In recent years, studies have shown that the tumour marker Neuron-specific enolase (NSE) is often elevated in CJD cases, however its diagnostic utility is primarily seen when combined with a test for the 14-3-3 protein. As of 2010, screening tests to identify infected asymptomatic individuals, such as blood donors, are not yet available, though methods have been proposed and evaluated.

In one third of patients with sporadic CJD, deposits of "prion protein (scrapie)," $PrP^{Sc}$, can be found in the skeletal muscle and/or the spleen. Diagnosis of vCJD can be supported by biopsy of the tonsils, which harbour significant amounts of PrPSc; however, biopsy of brain tissue is the definitive diagnostic test. Due to its invasiveness, biopsy will not be done if clinical suspicion is sufficiently high or low. A negative biopsy does not rule out CJD since it may predominate in a specific part of the brain



Spongiform change in CJD

The classic histologic appearance is spongiform change in the gray matter: the presence of many round vacuoles from one to 50 micrometres in the neuropil, in all six cortical layers in the cerebral cortex or with diffuse involvement of the cerebellar molecular layer. These vacuoles appear glassy or eosinophilic and may coalesce. Neuronal loss and gliosis are also seen. Plaques of amyloid-like material can be seen in the neocortex in new-variant CJD.

Unfortunately, vacuolization can be seen in other disease states. Diffuse cortical vacuolization occurs in Alzheimer's, and superficial cortical vacuolization occurs in ischemia and frontotemporal dementia. These vacuoles appear clear and punched-out. Larger vacuoles encircling neurons, vessels, and glia are a possible processing artifact.

- Clinical and Pathologic Characteristics:

| Characteristic | Classic CJD | Variant CJD |
|---|---|---|
| Median age at death | 68 years | 28 years |
| Median duration of illness | 4–5 months | 13–14 months |

| | | |
|---|---|---|
| Clinical signs and symptoms | Dementia; early neurologic signs | Prominent psychiatric/behavioral symptoms; painful dysesthesias;<br><br>delayed neurologic signs |
| Periodic sharp waves on electroencephalogram | Often present | Often absent |
| Signal hyperintensity in the caudate nucleus and putamen on diffusion-weighted and FLAIR MRI | Often present | Often absent |
| "Pulvinar sign" on MRI | Not reported | Present in >75% of cases |
| Immunohistochemical analysis of brain tissue | Variable accumulation. | Marked accumulation of protease-resistant prion protein |
| Presence of agent in lymphoid tissue | Not readily detected | Readily detected |
| Increased glycoform ratio on immunoblot analysis of<br><br>protease-resistant prion protein | Not reported | Marked accumulation of protease-resistant prion protein |
| Presence of amyloid plaques in brain tissue | May be present | May be present |

- An abnormal signal in the posterior thalami on T2- and diffusion-weighted images and fluid-attenuated inversion recovery sequences on brain magnetic resonance imaging (MRI); in the appropriate clinical context, this signal is highly specific for vCJD. (Source: CDC)

## *Treatment*

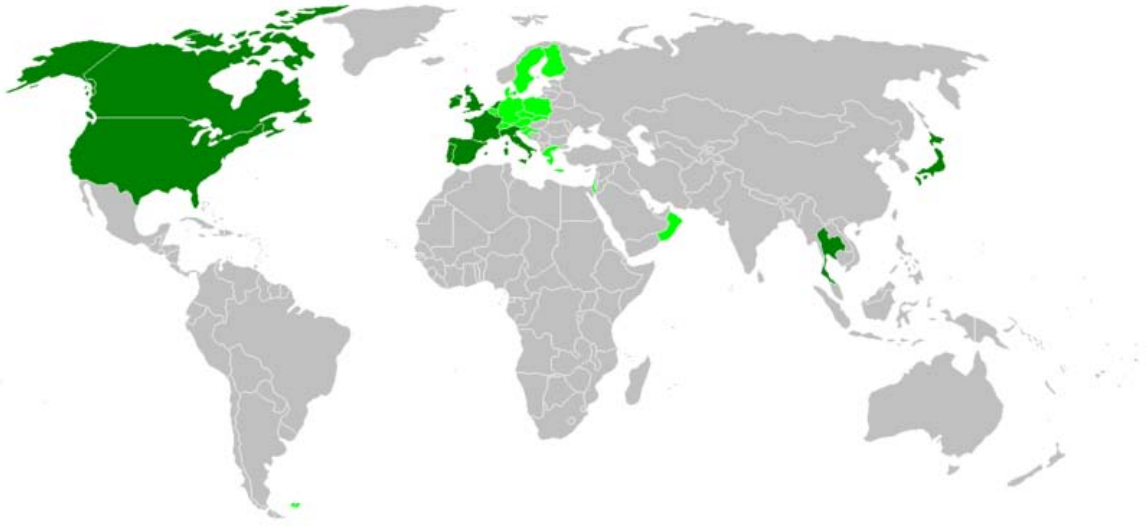
As of 2011 no generally accepted treatment for CJD exists; the disease is invariably fatal and research continues. An experimental treatment was given to a Northern Irish teenager, Jonathan Simms, beginning in January 2003. The medication, called pentosan polysulphate (PPS) and used to treat interstitial cystitis, is infused into the patient's lateral ventricle within the brain. PPS does not seem to stop the disease from progressing, and both brain function and tissue continue to be lost. However, the treatment is alleged to slow the progression of the otherwise untreatable disease, and may have contributed to the longer than expected survival of the seven patients who were studied. The CJD Therapy Advisory Group to the UK Health Departments advises that data are not sufficient to support claims that pentosan polysulphate is an effective treatment and suggests that further research in animal models is appropriate. A 2007 review of the treatment of 26 patients with PPS finds no proof of efficacy because of the lack of accepted objective criteria.

Scientists have investigated using RNA interference to slow the progression of scrapie in mice. The RNA blocks production of the protein that the CJD process transforms into prions. This research is unlikely to lead to a human therapy for many years.

Both amphotericin B and doxorubicin have been investigated as potentially effective against CJD, but as yet there is no strong evidence that either drug is effective. Further study has been taken with other medical drugs, but none are effective.

Dr. Michael Geschwind, Dr. Bruce Miller and Dr. Stanley Prusiner from University of California, San Francisco are currently running a treatment trial for sporadic CJD using quinacrine, a medicine originally created for malaria. Pilot studies showed quinacrine permanently cleared abnormal prion proteins from cell cultures, but results have not yet been published on the clinical study.

## *Epidemiology*



Dark green areas are countries that have confirmed human cases of variant Creutzfeldt-Jakob disease and light green are countries that have bovine spongiform encephalopathy cases.

Although CJD is the most common human prion disease, it is still rare, occurring in about one out of every one million people every year. It usually affects people aged 45–75, most commonly appearing in people between the ages of 60–65. The exception to this is the more recently-recognised 'variant' CJD (vCJD), which occurs in younger people.

CDC monitors the occurrence of CJD in the United States through periodic reviews of national mortality data. According to the CDC:

- CJD occurs worldwide at a rate of about 1 case per million population per year.
- On the basis of mortality surveillance from 1979 to 1994, the annual incidence of CJD remained stable at approximately 1 case per million persons in the United States.

- In the United States, CJD deaths among persons younger than 30 years of age are extremely rare (fewer than five deaths per billion per year).
- The disease is found most frequently in patients 55–65 years of age, but cases can occur in people older than 90 years and younger than 55 years of age.
- In more than 85% of cases, the duration of CJD is less than 1 year (median: four months) after onset of symptoms.

### New concerns

In *The Lancet* (June 2006), a University College London team suggested that it may take more than 50 years for vCJD to develop, from their studies of kuru, a similar disease in Papua New Guinea. The reasoning behind the claim is that kuru was possibly transmitted through cannibalism in Papua New Guinea when family members would eat the body of a dead relative as a sign of mourning. In the 1950s, cannibalism was banned. In the late 20th century, however, kuru reached epidemic proportions in certain Papua New Guinean communities, therefore suggesting that vCJD may also have a similar incubation period of 30 to 50 years. A critique to this theory is that while mortuary cannibalism was banned in Papua New Guinea in the 1950s, that does not necessarily mean that the practice ended. Fifteen years later Jared Diamond was informed by Papuans that the practice continued. Kuru may have passed to the Fore through the preparing of the dead body for burial.

These researchers noticed a genetic variation in some kuru patients that has been known to promote long incubation periods. They have also proposed that individuals who contracted CJD in the early 1990s represent a distinct genetic subpopulation, with unusually short incubation periods for BSE. This means that there may be many more vCJD patients who have longer incubation periods, which may surface many years later.

In 1997 a number of Kentuckians contracted the disease. It was discovered that all the victims had consumed squirrel brains.

## *History*

The disease was first described by German neurologist Hans Gerhard Creutzfeldt in 1920 and shortly afterwards by Alfons Maria Jakob, giving it the name Creutzfeldt–Jakob. Some of the clinical findings described in their first papers do not match current criteria for Creutzfeldt–Jakob disease, and it has been speculated that at least two of the patients in initial studies were suffering from a different ailment. An early description of familial CJD stems from the German psychiatrist and neurologist Friedrich Meggendorfer (1880 - 1953).

Chapter 9

# Bovine Spongiform Encephalopathy


Classic image of a cow with BSE. A feature of such disease is the inability of the infected animal to stand.

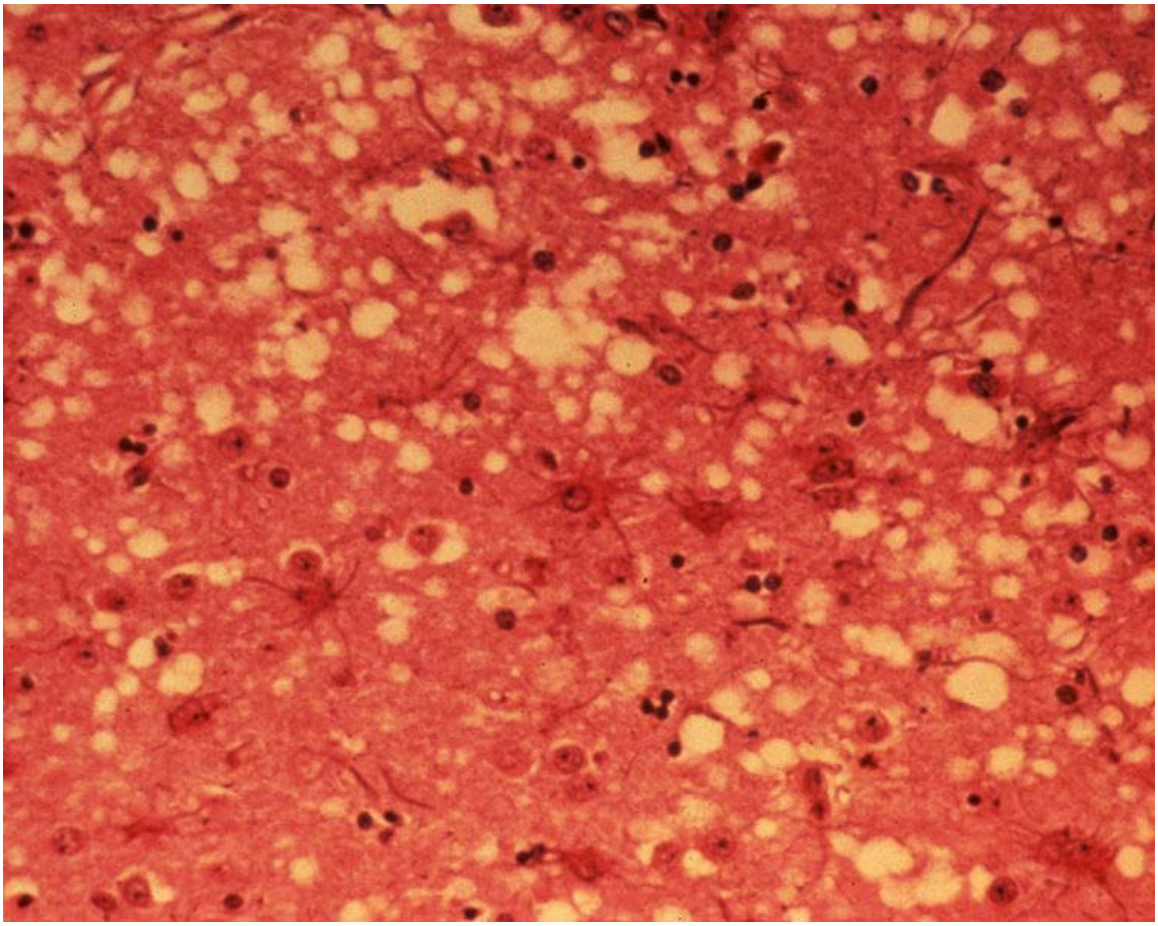
**Bovine spongiform encephalopathy** (**BSE**), commonly known as *mad-cow disease*, is a fatal neurodegenerative disease in cattle that causes a spongy degeneration in the brain and spinal cord. BSE has a long incubation period, about 30 months to 8 years, usually affecting adult cattle at a peak age onset of four to five years, all breeds being equally susceptible. In the United Kingdom, the country worst affected, more than 179,000 cattle have been infected and 4.4 million slaughtered during the eradication program.

The disease may be most easily transmitted to human beings by eating food contaminated with the brain or spinal cord or digestive tract of infected carcasses. However, it should also be noted that the infectious agent, although most highly concentrated in nervous tissue, can be found in virtually all tissues throughout the body, including blood. In humans, it is known as *new variant Creutzfeldt–Jakob disease* (vCJD or nvCJD), and by October 2009, it had killed 166 people in Britain (the most recent being of a different genotype than other sufferers), and 44 elsewhere with the number expected to rise because of the disease's long incubation period. Between 460,000 and 482,000 BSE-infected animals had entered the human food chain before controls on high-risk offal were introduced in 1989.

A British inquiry into BSE concluded that the epizootic was caused by cattle, who are normally herbivores, being fed the remains of other cattle in the form of meat and bone meal (MBM), which caused the infectious agent to spread. However, there are studies indicating that the cause and spread of BSE may be from the contamination of MBM from sheep with scrapie that were processed in the same slaughterhouse. The origin of the disease itself remains unknown. The infectious agent is distinctive for the high temperatures at which it remains viable; this contributed to the spread of the disease in Britain, which had reduced the temperatures used during its rendering process. Another contributory factor was the feeding of infected protein supplements to very young calves.

This first reported case in North America was in May 2003 from Alberta, Canada. The first known U.S. occurrence came in December of the same year. Canada announced two additional cases of BSE from Alberta in early 2005.

## *Cause*



Microscopic "holes" of tissue sections are examined in the lab. Source: APHIS

The infectious agent in BSE is believed to be a specific type of misfolded protein called a prion. Prion proteins carry the disease between individuals and cause deterioration of the brain. BSE is a type of transmissible spongiform encephalopathy (TSE). TSEs can arise in animals that carry an allele which causes previously normal protein molecules to contort by themselves from an alpha helical arrangement to a beta pleated sheet, which is the disease-causing shape for the particular protein. Transmission can occur when healthy animals come in contact with tainted tissues from others with the disease. In the brain these proteins cause native cellular prion protein to deform into the infectious state, which then goes on to deform further prion protein in an exponential cascade. This results in protein aggregates, which then form dense plaque fibers, leading to the microscopic appearance of "holes" in the brain, degeneration of physical and mental abilities, and ultimately death.

Different hypotheses exist for the origin of prion proteins in cattle. Two leading hypotheses suggest that it may have jumped species from the disease scrapie in sheep, or that it evolved from a spontaneous form of "mad cow disease" that has been seen occasionally in cattle for many centuries. Publius Flavius Vegetius Renatus records cases

of a disease with similar characteristics in the 4th and 5th century AD. The British Government enquiry took the view the cause was not scrapie as had originally been postulated, and was some event in the 1970s that it was not possible to identify.

Findings published in PLoS Pathogens (September 12, 2008) suggest that mad cow disease also is caused by a genetic mutation within a gene called Prion Protein Gene. The research shows, for the first time, that a 10-year-old cow from Alabama with an atypical form of bovine spongiform encephalopathy had the same type of prion protein gene mutation as found in human patients with the genetic form of Creutzfeldt–Jakob disease, also called genetic CJD for short. Besides having a genetic origin, other human forms of prion diseases can be sporadic, as in sporadic CJD, as well as foodborne. That is, they are contracted when people eat products contaminated with mad cow disease. This form of Creutzfeldt-Jakob disease is called variant CJD.

## *Epidemic in British cattle*

Cattle are normally herbivores. In nature, cattle eat grass. In modern industrial cattle-farming, various commercial feeds are used, which may contain ingredients including antibiotics, hormones, pesticides, fertilizers, and protein supplements. The use of meat and bone meal, produced from the ground and cooked left-overs of the slaughtering process as well as from the cadavers of sick and injured animals such as cattle, sheep, or chickens, as a protein supplement in cattle feed was widespread in Europe prior to about 1987. Worldwide, soya bean meal is the primary plant-based protein supplement fed to cattle. However, soya beans do not grow well in Europe, so cattle raisers throughout Europe turned to the less expensive animal by-product feeds as an alternative. A change to the rendering process in the early 1980s may have resulted in a large increase of the infectious agents in the cattle feed. A contributing factor was suggested to have been a change in British laws that allowed a lower temperature sterilization of the protein meal. While other European countries like Germany required said animal byproducts to undergo a high temperature steam boiling process, this requirement had been eased in Britain as a measure to keep prices competitive. Later the British Inquiry dismissed this theory saying "changes in process could not have been solely responsible for the emergence of BSE, and changes in regulation were not a factor at all."

The first confirmed animal to fall ill with the disease occurred in 1986 in Britain, lab tests the following year indicated the presence of BSE; it was only in November 1987 that the British Ministry of Agriculture accepted it had a new disease on its hands. Subsequently, 165 people (up until October 2009) acquired and died of a disease with similar neurological symptoms subsequently called vCJD, or (new) variant Creutzfeldt-Jakob disease. This is a separate disease from 'classical' Creutzfeldt–Jakob disease, which is not related to BSE and has been known about since the early 1900s. Three cases of vCJD occurred in people who had lived in or visited Britain — one each in Ireland, Canada and the United States. There is also some concern about those who work with (and therefore inhale) cattle meat and bone meal, such as horticulturists, who use it as fertilizer. Up to date statistics on all types of CJD are published by the National Creutzfeldt-Jakob Disease Surveillance Unit (NCJDSU) in Edinburgh.

For many of the vCJD patients, direct evidence exists that they had consumed tainted beef, and this is assumed to be the mechanism by which all affected individuals contracted it. Disease incidence also appears to correlate with slaughtering practices that led to the mixture of nervous system tissue with hamburger and other beef. It is estimated that 400,000 cattle infected with BSE entered the human food chain in the 1980s. Although the BSE epizootic was eventually brought under control by culling all suspect cattle populations, people are still being diagnosed with vCJD each year (though the number of new cases currently has dropped to fewer than 5 per year). This is attributed to the long incubation period for prion diseases, which are typically measured in years or decades. As a result the extent of the human vCJD outbreak is still not fully known.

The scientific consensus is that infectious BSE prion material is not destroyed through normal cooking procedures, meaning that contaminated beef foodstuffs prepared "well done" may remain infectious.

In 2004, researchers reported evidence of a second contorted shape of prions in a rare minority of diseased cattle. If valid, this would imply a second strain of BSE prion. Very little is known about the shape of disease-causing prions, because their insolubility and tendency to clump thwarts application of the detailed measurement techniques of structural biology. But cruder measures yield a "biochemical signature" by which the newly discovered cattle strain appears different from the familiar one, but similar to the clumped prions in humans with traditional CJD Creutzfeldt-Jakob Disease. The finding of a second strain of BSE prion raises the possibility that transmission of BSE to humans has been underestimated, because some of the individuals diagnosed with spontaneous or "sporadic" CJD may have actually contracted the disease from tainted beef. So far nothing is known about the relative transmissibility of the two disease strains of BSE prion.

Alan Colchester, a professor of neurology at the University of Kent, and Nancy Colchester, writing in the September 3, 2005 issue of the medical journal *The Lancet*, proposed a theory that the most likely initial origin of BSE in Britain was the importation from the Indian subcontinent of bone meal which contained CJD infected human remains. The government of India vehemently responded to the research calling it "misleading, highly mischievous; a figment of imagination; absurd," further adding that India maintained constant surveillance and had not had a single case of either BSE or vCJD. The authors responded in the January 22, 2006 issue of The Lancet that their theory is unprovable only in the same sense as all other BSE origin theories are and that the theory warrants further investigation.

## Epizootic in the United Kingdom

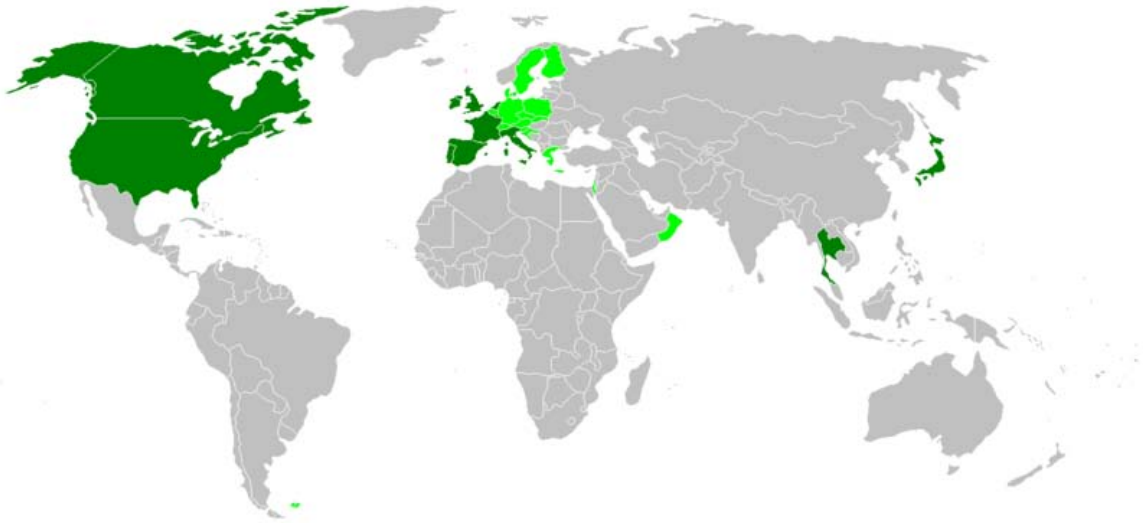
During the course of the investigation into the BSE epizootic, an enquiry was also made into the activities of the Department of Health and its Medicines Control Agency. On May 7, 1999, in his written statement to the BSE Inquiry, David Osborne Hagger reported on behalf of the Medicines Control Agency that in a previous enquiry the Agency had been asked to:

*"... identify relevant manufacturers and obtain information about the bovine material contained in children's vaccines, the stocks of these vaccines and how long it would take to switch to other products."* It was further reported that the: *"... use of bovine insulin in a small group of mainly elderly patients was noted and it was recognised that alternative products for this group were not considered satisfactory."* A medicines licensing committee report that same year recommended that: *"... no licensing action is required at present in regard to products produced from bovine material or using prepared bovine brain in nutrient media and sourced from outside the United Kingdom, the Channel Isles and the Republic of Ireland provided that the country of origin is known to be free of BSE, has competent veterinary advisers and is known to practise good animal husbandry."* In 1990 the British Diabetic Association became concerned regarding the safety of bovine insulin and the government licensing agency assured them that: *"... there was no insulin sourced from cattle in the Britain or Ireland and that the situation in other countries was being monitored."* In 1991 a European Community Commission: *"... expressed concerns about the possible transmission of the BSE/scrapie agent to man through use of certain cosmetic treatments."* Sources in France reported to the British Medicines Control Agency: *"... that there were some licensed surgical sutures derived from French bovine material."* Concerns were also raised: *"... regarding a possible risk of transmission of the BSE agent in gelatin products*."

## *Epidemiology*

| Country | BSE cases | vCJD cases |
|---|---|---|
| Austria | 5 | 0 |
| Belgium | 133 | 0 |
| Canada | 17 | 1 |
| Czech Republic | 28 | 0 |
| Denmark | 14 | 0 |
| Falkland Islands | 1 | 0 |
| Finland | 1 | 0 |
| France | 900 | 25 |
| Germany | 312 | 0 |
| Greece | 1 | 0 |
| Hong Kong | 2 | 0 |
| Ireland | 1,353 | 4 |
| Israel | 1 | 56 |
| Italy | 138 | 2 |
| Japan | 26 | 1 |
| Liechtenstein | 2 | 0 |
| Luxembourg | 2 | 1 |
| Netherlands | 85 | 3 |
| Oman | 2 | 0 |

| | | |
|---|---|---|
| Poland | 21 | 0 |
| Portugal | 875 | 2 |
| Saudi Arabia | 0 | 1 |
| Slovakia | 15 | 0 |
| Slovenia | 7 | 0 |
| Spain | 412 | 5 |
| Sweden | 1 | 0 |
| Switzerland | 453 | 0 |
| Thailand | | 2 |
| United Kingdom | 183,841 | 170 |
| United States | 3 | 3 |
| **Total** | **188,579** | **275** |



Dark green areas are countries with confirmed human cases of vCJD. Light green shows countries which have reported cases of only BSE.

The table to the right summarizes reported cases of BSE and of vCJD by country. BSE is the disease in *cattle*, while vCJD is the disease in *people*.

The tests used for detecting BSE vary considerably as do the regulations in various jurisdictions for when, and which cattle, must be tested. For instance, in the EU the cattle tested are older (30 months+), while many cattle are slaughtered earlier than that. At the opposite end of the scale, Japan tests all cattle at the time of slaughter. Tests are also difficult as the altered prion protein has very low levels in blood or urine, and no other signal has been found. Newer tests are faster, more sensitive, and cheaper, so it is possible that future figures may be more comprehensive. Even so, currently the only reliable test is examination of tissues during an autopsy.

It is notable that there are no cases reported in Argentina, Australia, Brazil, New Zealand, Uruguay, and Vanuatu where cattle are mainly fed outside on grass pasture and, mainly in Australia, non-grass feeding is done only as a final finishing process before the animals are processed for meat.

As for vCJD in humans, autopsy tests are not always done and so those figures too are likely to be too low, but probably by a lesser fraction. In the United Kingdom anyone with possible vCJD symptoms must be reported to the *Creutzfeldt-Jakob Disease Surveillance Unit*. In the United States, the CDC has refused to impose a national requirement that physicians and hospitals report cases of the disease. Instead, the agency relies on other methods, including death certificates and urging physicians to send suspicious cases to the National Prion Disease Pathology Surveillance Center (NPDPSC) at Case Western Reserve University in Cleveland, which is funded by the CDC.

## *Practices in the United States relating to BSE*

Soybean meal is cheap and plentiful in the United States. The Million and a half tons of Cotton seed meal produced in the US every year that is not suitable for humans or any simple stomach animals is even cheaper than soybean meal. It is just as nutritious and cattle eat it just as well if not better than Soybean meal. Historically meat and bone meal, blood meal and meat scraps have almost always commanded a higher price as a feed additive than oil seed meals in the US so there was never much incentive to use animal products to feed to ruminants. As a result, the use of animal byproduct feeds was never common, as it was in Europe. However, U.S. regulations only partially prohibit the use of animal byproducts in feed. In 1997, regulations prohibited the feeding of mammalian byproducts to ruminants such as cows and goats. However, the byproducts of ruminants can still be legally fed to pets or other livestock such as pigs and poultry such as chickens. In addition, it is legal for ruminants to be fed byproducts from some of these animals. A proposal to end the use of cow blood, restaurant scraps, and poultry litter (fecal matter, feathers) in January 2004 has yet to be implemented.

### Regulatory failures

In February 2001, the USGAO reported that the FDA, which is responsible for regulating feed, had not adequately policed the various bans. Compliance with the regulations was shown to be extremely poor before the discovery of the Washington cow, but industry representatives report that compliance is now 100%. Even so, critics call the partial prohibitions insufficient. Indeed, US meat producer Creekstone Farms alleges that the USDA is preventing BSE testing from being conducted.

The USDA has issued recalls of beef supplies that involved introduction of downer cows into the food supply. Hallmark/Westland Meat Packing Company was found to have used electric shocks to prod downer cows into the slaughtering system in 2007. Possibly due to pressure from large agribusiness, the United States has drastically cut back on the number of cows inspected for BSE.

## The ban on British beef

The BSE crisis led to the European Union banning exports of British beef with effect from March 1996; the ban would last for 10 years before it was finally lifted on 1 May 2006, despite attempts in May 1996 by British prime minister John Major to get the ban lifted.

If indeed a form of Bovine Spongiform Encephalopathy (BSE) exists in the United States, one might expect to see a rise in the number of cases of Creutzfeldt-Jakob disease (CJD). CJD, however, is not a reportable illness in the United States (Holman, 1995). Because the Centers for Disease Control (CDC) does not actively monitor the disease (Altman, 1996d) a rise similar to the one in Britain could be missed (Altman, 1996d). Already, a number of U.S. CJD clusters have been found. In the largest known U.S. outbreak of sporadic cases to date (Flannery, 1996) a fivefold expected rate was found to be associated with cheese consumption in Pennsylvania's Lehigh Valley (Little, 1993) A striking increase in CJD was also reported in Florida (Berger, 1994) and there is an anecdotal report of an cluster in Oregon (Boule, 1996). An analysis of death certificates in a number of states, though, showed an overall stable and typical CJD incidence rate from 1979 to 1993 (World, 1996). To track the disease, the CDC has just initiated a four-state study of death certificates (Altman, 1996a), but since it is considered well known that death-certificate diagnoses are not always accurate (Davanpour, 1993) the survey may not provide an accurate assessment.

The true prevalence of prion diseases in the United States or any other country remains a mystery (Harrison, 1991). Compounding the uncertainty, autopsies are rarely performed on atypical dementias (Harrison, 1991), because medical professionals fear infection (Altman, 1996a). The officially reported rate in this country is less than 1 case in a million people per year (World, 1996). An informal survey of neuropathologists, however, registered a theoretical range of 2-12% of all dementias as actually CJD (Harrison, 1991). And hundreds of thousands of Americans suffer from severe dementias every year (Brayne, 1994; United, 1995). Two other studies average about a 3% CJD rate among dementia patients (Mahendra, 1987; Wade, 1987). A preliminary 1989 University of Pennsylvania study showed that 5% of patients diagnosed with dementia were actually dying from Creutzfeldt-Jakob disease (Boller, 1989). It would seem CJD is seriously underdiagnosed at present (Harrison, 1991).

The most common misdiagnosis of CJD is Alzheimer's disease (Harrison, 1991). CJD was even described by the top CJD researcher of the government of the United States (Wlazelek, 1990a) as "Alzheimer's in fast forward (Wlazelek, 1990b)." The symptoms and pathology of both diseases overlap (Brown, 1989). There can be spongy changes in Alzheimer's, for example, and senile plaques in CJD (Brown, 1989). The causes may overlap as well; epidemiological evidence suggests that people eating meat more than four times a week for a prolonged period have a three times higher chance of suffering a dementia than long-time vegetarians (Giem, 1993), although this result may be confounded by vascular factors (Van Duijn, 1996).

Paul Brown, medical director for the U.S. Public Health Service (Gruzen, 1996), said that the brains of the young people who died from the new CJD variant in Britain even look like Alzheimer's brains (Hager, 1996). Stanley Prusinger, the scientist who coined the term prion, speculates Alzheimer's may in fact turn out to be a prion disease (Prusiner, 1984). In younger victims the disease could look like multiple sclerosis or a severe viral infection, according to Alzheimer's expert Gareth Roberts (Brain, 1996).

An estimated two to three million Americans are afflicted by Alzheimer's (Scully, 1993); it is the fourth leading cause of death among the elderly in the U.S (Perry, 1995). Twenty percent or more of people clinically diagnosed with Alzheimer's disease are found at autopsy to not have had Alzheimer's at all (McKhann, 1984). At Yale, out of 46 patients clinically diagnosed with Alzheimer's, 6 were proven to be CJD at autopsy (Manuelidis, 1989). In another post-mortem study 3 out of 12 "Alzheimer" patients actually died from a spongiform encephalopathy (Teixeira, 1995).

Carleton Gajdusek, who was awarded a Nobel Prize for his work with prion diseases (Manuelidis, 1985), estimates that 1% of people showing up in Alzheimer clinics actually have CJD (Folstein, 1983). That means that hundreds of people (Hoyert, 1996; United, 1995) may already be dying from mad cow disease each year in the United States.

## Effect on the U.S. beef industry

Japan was the top importer of U.S. beef, buying 240,000 tons valued at $1.4 billion in 2003. After the discovery of the first case of BSE in the U.S. on December 23, 2003, Japan stopped U.S. beef imports in December 2003. In December 2005, Japan once again allowed imports of U.S. beef, but reinstated its ban in mid-January 2006 after a technical violation of the U.S.-Japan beef import agreement: a vertebral column, which should have been removed prior to shipment, was included in a shipment of veal.

Tokyo yielded to U.S. pressure to resume imports, ignoring consumer worries about the safety of U.S. beef, said Japanese consumer groups. Michiko Kamiyama from Food Safety Citizen Watch and Yoko Tomiyama from Consumers Union of Japan said about this: "The government has put priority on the political schedule between the two countries, not on food safety or human health."

65 nations implemented full or partial restrictions on importing U.S. beef products because of concerns that U.S. testing lacked sufficient rigor. As a result, exports of U.S. beef declined from 1,300,000 metric tons in 2003, before the first mad cow was detected in the US, to 322,000 metric tons in 2004. This has increased since then to 771,000 metric tons in 2007.

On December 31, 2006, Hematech, Inc, a biotechnology company based in Sioux Falls, South Dakota, announced that it had used genetic engineering and cloning technology to produce cattle that lacked a necessary gene for prion production - thus theoretically making them immune to BSE.