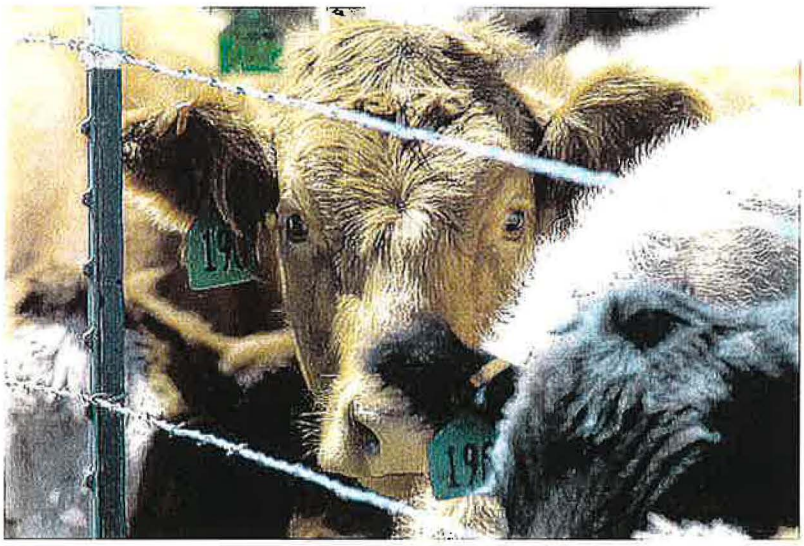
ENDOGENOUS BIOTERRORISM



Diseases of Protein Misfolding

- Creutzfeld-Jakob Disease
- Gerstmann-Sträussler-Scheinker Disease
- Kuru
- Scrapie
- Bovine Spongiform Encephalopathy
- Chronic Wasting Disease
- Transmissible Mink Encephalopathy
- Feline Spongiform Encephalopathy
- Exotic Spongiform Encephalopathy
  - Kudu
  - Nyala
  - Oryx
  - Eland
  - Gemsbok
  - Cheetah
  - Ocelot
  - Puma
  - Bison
  - Tiger

John M. Dietschy M. D.
University of Texas Southwestern Medical School

John M. Dietschy, M.D.

Dr. Dietschy is professor of Internal Medicine in the Division of Digestive and Liver Diseases. His research interests are focused on the control of cholesterol metabolism in the body and in the central nervous system.

This is to acknowledge that Dr. Dietschy has disclosed no financial interests or other relationships with commercial concerns related to this program. He will not be discussing "off-label" uses in his presentation.

A BRIEF CHRONOLOGY OF PRION DISEASE

(Modified from "How the Cows Turned Mad" by Maxime Schwartz, 2003)

1740-1760	Earliest Descriptions of Scrapie
1870-1880	Pasteur and Koch Demonstrate Role of Microbes in Disease
1898	Spongiform Pathology Described in Scrapie
1918	Scrapie Found To Be Contagious
1920-1923	First Cases of Human Disease Described by Creutzfeldt and Jakob
1936-1938	Scrapie Demonstrated To Be Transferable by Inoculation
1955-1957	Kuru Described among Fore People of Papua New Guinea
1957	Similarity between Kuru and CJD Noted
1959	Similarity between Kuru and Scrapie Noted
1961	Scrapie Successfully Transmitted to Mice
1963-1966	Brain Extracts from Patients with Kuru and CJD and from Scrapie Cause Disease in Primates
1967	Hypothesis that Infectious Agent Was a Protein
1976	Nobel Prize Awarded Gajdusek
1979	CJD Shown To Have Constant Incidence around the World
1980	Kuru and CJD Transmitted to Monkeys through Feeding Brain
1982	The Proteinaceous Infectious Agent First Named a "Prion"
1985	Prion Protein Showed To Be a Normal Protein in all Mammals
1987	First Article on Bovine Spongiform Encephalopathy
1988	MBM Shown to Transmit Bovine Spongiform Encephalopathy
1989	A Subset of CJD Patients Shown To Have Mutations in Prion Protein
1989-1990	Infectious Prion Postulated To Have Abnormal Configuration
1992-1993	Deletion of Normal Prion Protein in a Species Shown to Prevent Disease Induced by Infectious Agent
1993	Infectious Prion Shown To Have High Content of Beta-Sheets
1994	First Description of "Strains" of Prions
1996	Ten Cases of Human vCJD Reported in England
1997	Nobel Prize Awarded Prusiner
1997-2000	First Description of the Role of Lymphatic System in the Transmission of Prions
2000-2004	Recognition of a Rapidly Spreading Form of Prion Disease in Elk and Deer in the United States
2004	First Case of vCJD from Blood Transfusion

I. INTRODUCTION

This review deals with recent information concerning the pathogenesis of a group of illnesses that are generally referred to as spongiform encephalopathies that result when one of the normal proteins in the body misfolds. This syndrome is seen in many mammalian species, including humans. In humans, the disease is commonly referred to as Creutzfeldt-Jakob disease, and this disorder can be familial, sporadic or acquired. This review will concentrate on the sporadic and acquired forms of spongiform encephalopathy in both animals and humans.

II. THE PROTEIN INVOLVED

This set of diseases is unique in that they can be both familial and infectious, and, further, the agent responsible for the diseases does not contain nucleic acids. Rather, these disorders occur when a normal protein in the body becomes misfolded. This prion protein is one of thousands of proteins synthesized each day in many organs of all mammals and humans. This normal protein is usually indicated by the term PrP^c. The gene for this protein is expressed in many tissues in the body including cells of the central nervous system, peripheral nerves and lymphocytes. The protein has approximately 200 amino acids and a molecular weight of about 30 kD. It is synthesized in the endoplasmic reticulum, processed in the Golgi apparatus and transported to the cell membrane. There it is attached to the outside of the membrane through a GPI anchor inserted into cholesterol-rich rafts. The protein also apparently cycles back into the cell through clathrin coated pits in a manner that is similar to the behavior of the LDL receptor. Ironically, despite all of the work that has been done over the past 25 years, the normal function of this protein is not understood. Its behavior is similar to that of various receptor molecules and it contains two high affinity sites for copper ions. Conceivably, it is involved in maintaining copper balance within various tissues. However, at this time its actual function is not understood. When PrP^c is knocked out, there is essentially no phenotype.

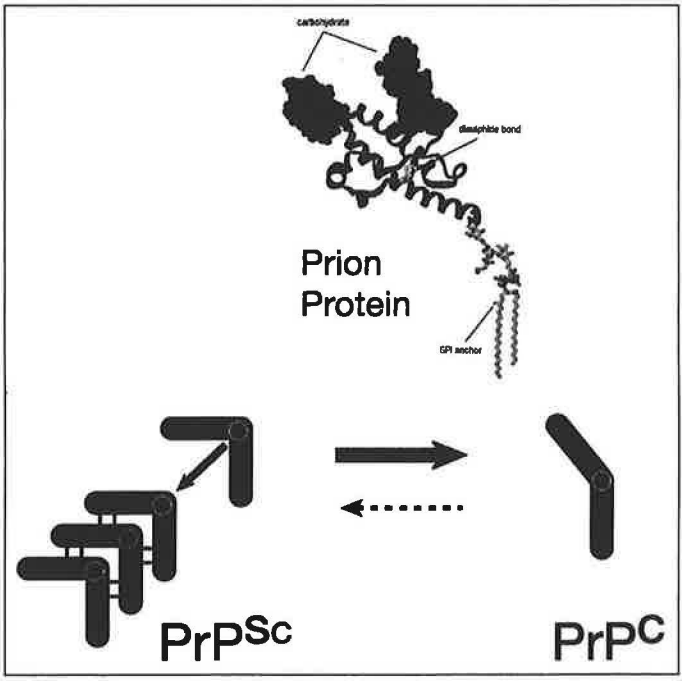


Figure 1

As seen in **Fig. 1**, the unique feature of this protein is that under certain conditions it can become folded into a different configuration that is resistant to cellular proteases and that is capable of forming insoluble polymeric complexes. This abnormal form of the prion protein is designated PrP^{Sc}. The term prion was coined in 1982 to describe a "proteinatious infectious particle." The superscript "c" stands for cellular (the normal cellular prion protein) while the superscript "Sc" stands for the sheep disease scrapie. When large amounts of PrP^{Sc} are found in a given tissue the insoluble molecules form plaques similar to those found in other diseases such as Alzheimer's disease.

The gold standard for the identification of these diseases is the finding of protease-resistant accumulation of the PrP^{Sc} in the brain, nerves or reticulolymphatic system. As illustrated in **Fig. 2**, the normal protein, PrP^{c}, can be identified in normal tissue (lane 1) but is totally destroyed when treated with the protease (lane 2). In contrast, the abnormal protein, PrP^{Sc}, runs with the same molecular weight (lane 3) but is not destroyed by protease treatment (lane 4). It should be emphasized that PrP^{c} and PrP^{Sc} have exactly the same molecular weight and the same amino acid structure. The only difference is that PrP^{Sc} has been folded differently, contains a large proportion of β-sheets, is insoluble and is resistant to protease treatment. The critical problem in these diseases is to understand the forces that lead to this abnormal folding of the PrP^{c} protein.

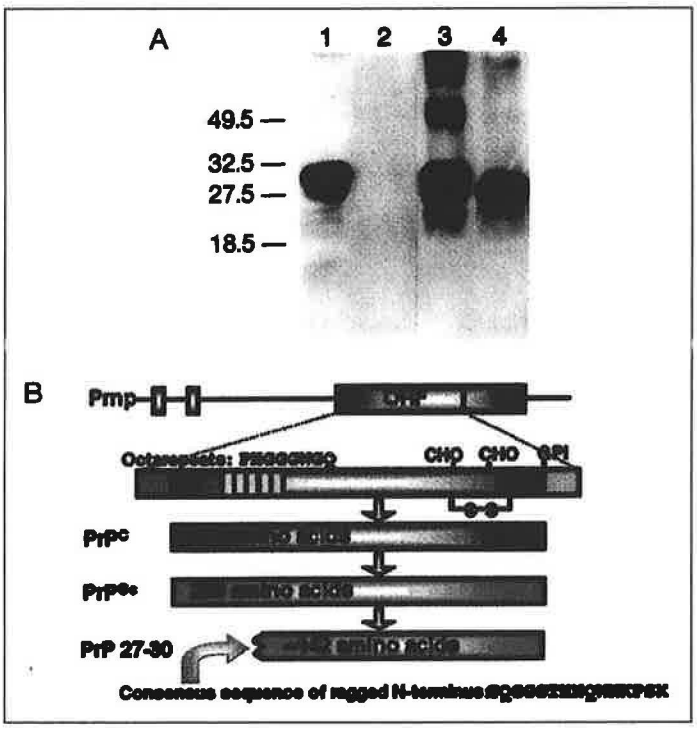


Figure 2

III. THE CHARACTERISTICS OF SPONGIFORM ENCEPHALOPATHY

When excessive amounts of the abnormal PrP^{Sc} protein are deposited in plaques in the brain there is widespread destruction of neurons in many regions. The exact location of these lesions may vary according to the strain of prion that is being formed. This destruction leads to a variety of symptoms that affect emotions, cognitive function and motor function. The symptom complex is similar in animals and humans with this disorder. Initially, affected animals will show changes in behavior and this will be followed by obvious neurological dysfunction, inability to eat and, finally, death. Similarly, in humans there may be relatively abrupt onset of changes in behavior and mentation. These early symptoms, for example, may include anxiety, irritability, insomnia, memory loss, impaired concentration and sudden loss of ability to perform well known tasks. These symptoms may rapidly progress to confusion, loss of appetite, depression, panic attacks and suicidal ideation. These symptoms are rapidly accompanied by motor dysfunction including tremors, impaired balance, paralysis, inability to swallow, and, finally, death. In humans this sequence of development seldom lasts longer than 10-12 months. This syndrome can be seen in young individuals (10-30 years of age) in various acquired forms of Creutzfeldt-Jakob disease (CJD), in individuals who are 40-55 years of age (familial CJD) and in older individuals (55-70 years of age) (sporadic CJD). Similarly, spongiform encephalopathy becomes symptomatic in older sheep (approximately 2 years of age) and older cows (5-7 years of age).

IV. NORMAL PROTEIN FOLDING

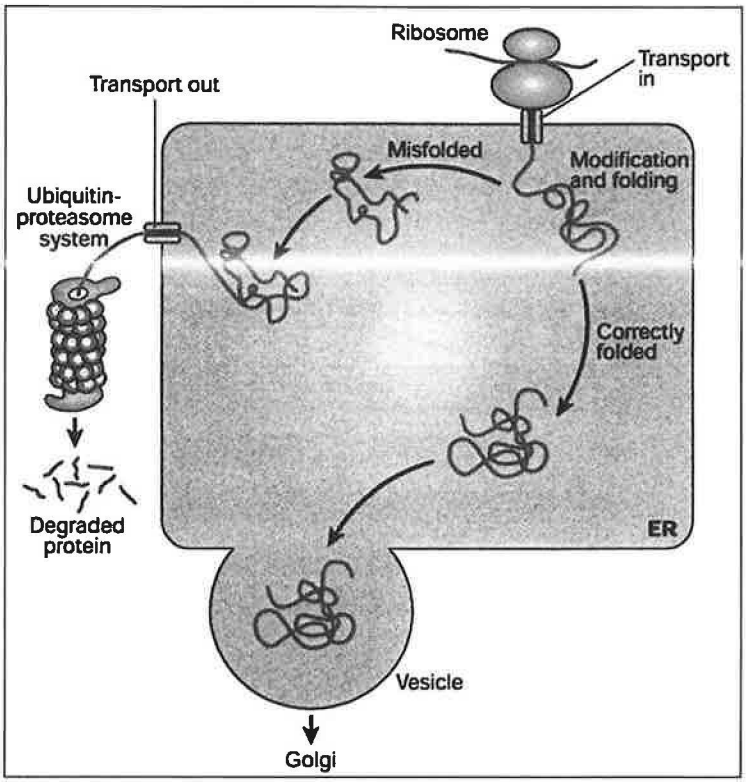


Figure 3

In order to understand the pathogenesis of these diseases, it is necessary to understand the normal mechanisms that result in the folding of nascent proteins into their functional, native form. As illustrated in **Fig. 3**, the cell has mechanisms for testing whether or not a particular protein is correctly folded and, if not, for destroying that abnormally folded molecule. In this scheme proteins are synthesized by the ribosome in the endoplasmic reticulum. If the protein has achieved its functional, native configuration it will then be transported to the Golgi for further processing and then delivered to the sites within the cell or on the cell membrane where it normally functions. If the endoplasmic reticulum, however, senses that the protein is incorrectly folded, then mechanisms exist for moving this misfolded protein out of the endoplasmic reticulum and degrading it under the action of various proteases.

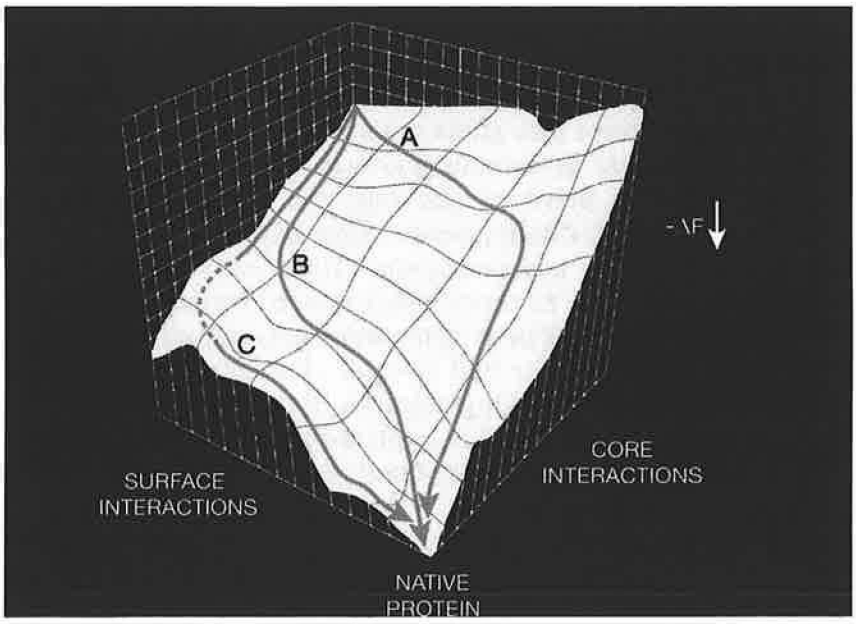


Figure 4

However, more recent work suggests that this folding process is more complex. As illustrated in **Fig. 4**, the folding of a large, complex polypeptide chain can follow many different pathways. In this illustration, one pathway (A) may involve initially interaction of surface components of the peptide followed later by core interactions. Alternatively (B), core interactions may precede those on the surface of the protein. In either case, the folded protein has achieved a stable, functional configuration. In some cases, accessory proteins, i.e., chaperones, may assist in the folding process and prevent the formation of an abnormal form. For example, as also illustrated

in this figure (C), a chaperone may prevent the folding of the protein into a nonfunctional form by preventing its movement into an energy sink from which it cannot refold.

As illustrated in **Fig. 5**, such a chaperone (here designated as "protein X") may interact with the normal prion protein and convert it into an abnormally folded form. In this illustration, protein X interacts with a small portion of PrP^c, raises its energy level slightly, and allows it to unfold and then refold as PrP^{Sc}. This process may be ongoing throughout the lifetime of most mammals but is fundamentally of little consequence since the equilibrium between PrP^{Sc} and PrP^c is normally overwhelmingly in the direction of formation of the normal protein. Understanding the prion associated diseases, therefore, involves understanding the processes that may alter this equilibrium and allow for the formation of significant amounts of PrP^{Sc} within the brain and other tissues of the animal or human.

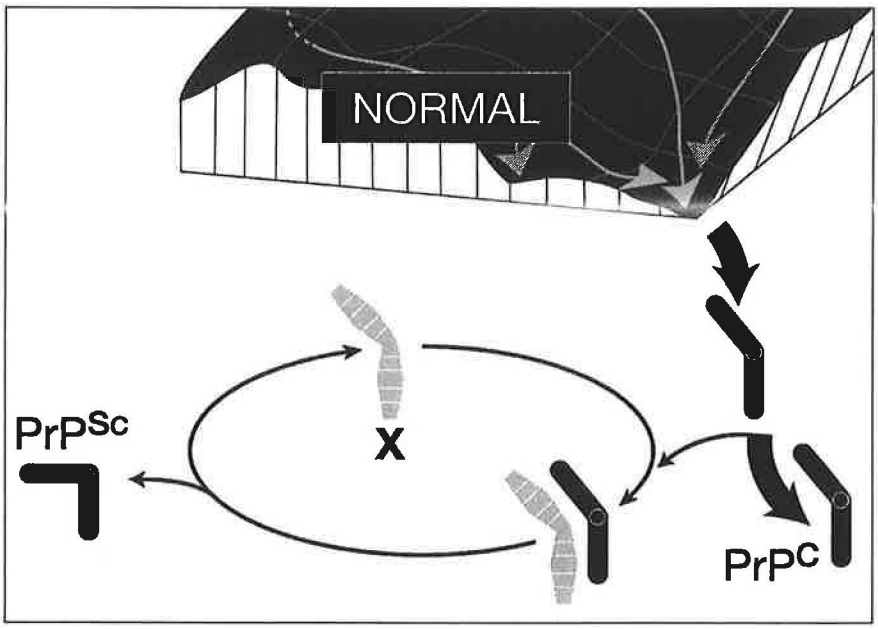


Figure 5

V. PRINCIPLES DESCRIBING PRION ASSOCIATED DISEASE

The characteristics of prion associated disease are very different from those found with other genetic diseases or with a variety of infectious diseases, yet prion disease has characteristics of both a familial and infectious disease.

The first general principle has to do with the characteristics of the infectious agent. As illustrated in **Fig. 6**, the particle that transmits the disorder is the misfolded prion protein. There is no evidence for RNA or DNA associated with this protein particle. Furthermore, there is no change in the amino acid sequence in the protein (except in the familial forms of CJD). The sole abnormality is that the PrP^c has been converted to PrP^{Sc}, and this results in resistance to protease degradation and promotes aggregation into multimers.

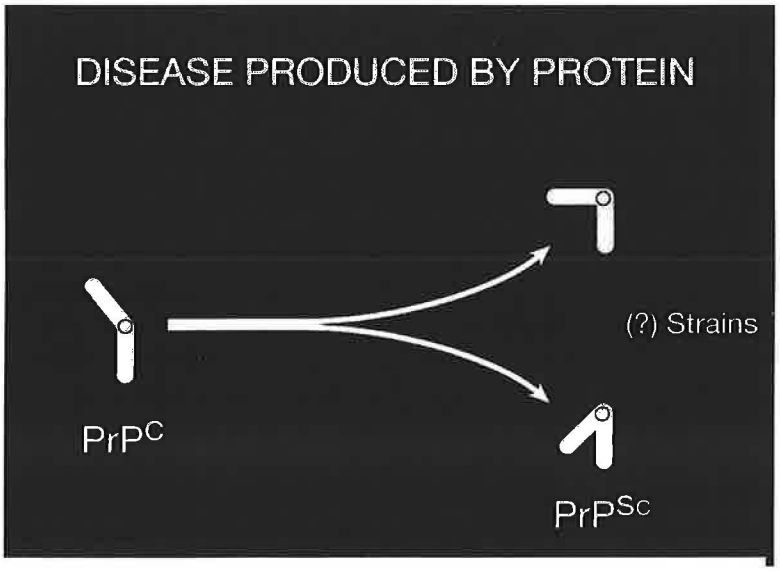


Figure 6

Within the same species, there can also be different "strains" of prions. These differences again apparently arise from other configurations of the misfolded protein and not from any change in amino acid structure. In this manner, the same protein, misfolded into different configurations, can give rise to slightly different clinical syndromes by attacking slightly different regions of the central nervous system.

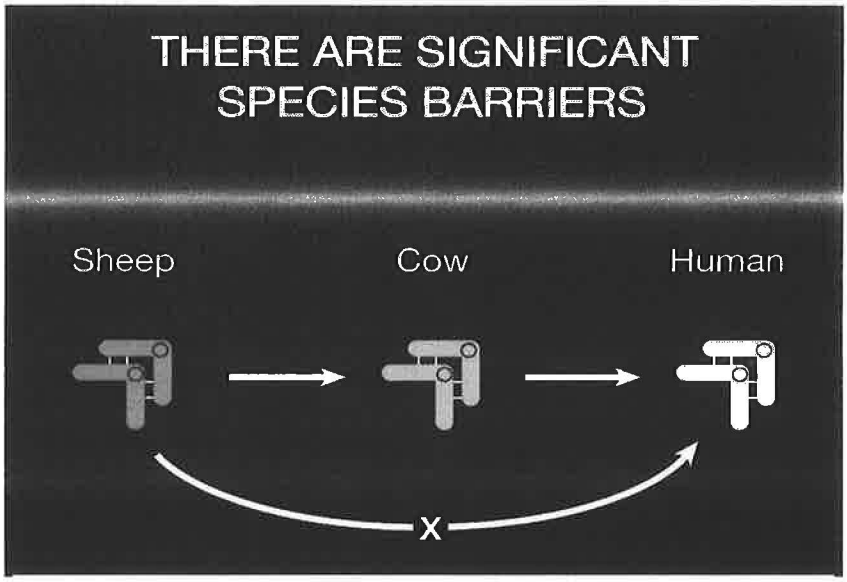


Figure 7

A second general principle is illustrated in **Fig. 7**. There are clearly significant species barriers to the transmission of these diseases from one species to another. The molecular basis for these barriers is not well understood at this time but presumably somehow involves the degree of "relatedness" of the prion protein from the different species. There is currently little doubt that the sheep prion can be transmitted to the cow and produce spongiform encephalopathy. Similarly, it is now established that the cow prion can be transmitted to humans and produce acquired Creutzfeldt-Jakob disease. It is also well established, however, that the sheep prion cannot be transmitted directly to humans. The molecular mechanisms responsible for this barrier remain to be elucidated.

Another general principle of prion disease, illustrated in **Fig. 8**, is that the genetic characteristics of the prion protein in the recipient dictates whether or not an exogenous prion will initiate disease. This principle is particularly important with respect to acquired CJD disease in humans. As shown in this figure, the normal human prion protein, PrP^{c} , contains a number of polymorphisms, particularly at position 129. At this position, the majority of humans are heterozygous and have the two amino acids M and V. A lesser percent of the population is homozygous at position 129 (M129M). Apparently, this genetic makeup at position 129 determines whether the normal human prion protein can be induced to refold into an abnormal form by an exogenous PrP^{Sc}. As illustrated in this figure, humans who are heterozygous at position 129 will not develop spongiform encephalopathy if infected with an exogenous PrP^{Sc}. In contrast, individuals who are homozygous (M129M) are

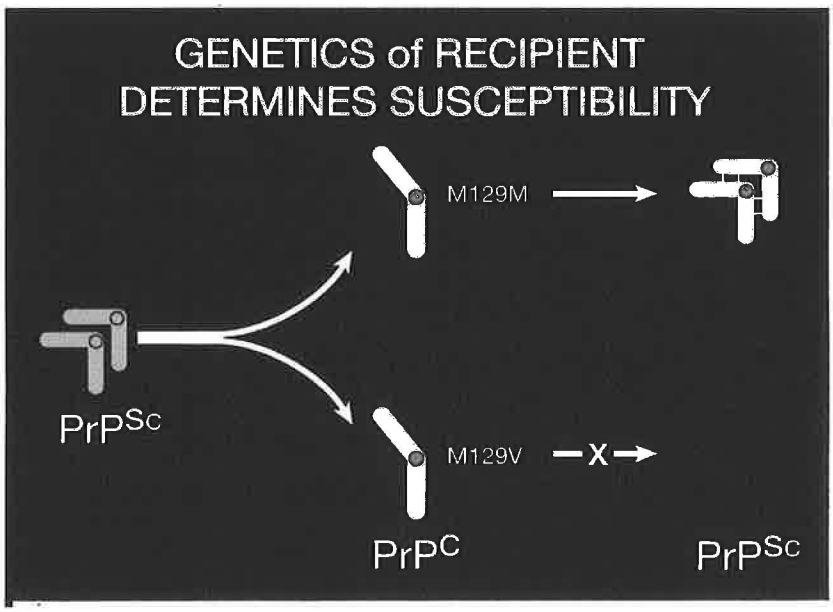


Figure 8

susceptible to acquiring this disease if infected with the exogenous prion. This genetic effect is so profound that in the recent British epidemic of prion disease, all individuals who developed the clinical syndrome were homozygous at the 129 position even though the majority of the British population is heterozygotes.

Figure 9

The fourth general principle is shown in **Fig. 9**. When spongiform encephalopathy develops in a human, it is because that individual's own normal prion protein has been reconfigured into the PrP^{Sc} form. This is true even though the original disease may have been induced by an exogenous prion that came from a different species. While this exogenous prion may have initiated the auto-catalytic process, the disease is caused by converting that patient's own normal prion protein into the abnormal configuration. As a consequence, the disease-causing entity is recognized by the body as "self" and so elicits no inflammatory reaction, no fever and no pleocytosis. Worst of all, these abnormal prions elicit no serological response. Thus, individuals with spongiform encephalopathy have no secondary signs of tissue destruction and do not develop any kind of diagnostic serological change.

VI. TYPES OF HUMAN SPONGIFORM ENCEPHALOPATHY

Human disease resulting from misfolded prion protein can be due to three different causes. These include the uncommon situation in which an inherited mutation is present in PrP^{c} (inherited Creutzfeldt-Jakob disease), the situation where the disease apparently occurs spontaneously (sporadic Creutzfeldt-Jakob disease) and the situation in which an exogenous prion has been acquired from another human or another species (acquired Creutzfeldt-Jakob disease). There are now at least 20-30 different mutations that can result in inherited spongiform encephalopathy in the human. In general, these inherited diseases are manifest clinically with a very high penetrance and in relatively young individuals. This syndrome will not be discussed further in this protocol.

The most common form of this disease in humans is spongiform encephalopathy that apparently occurs for no reason or, in other terms, is sporadic. In general, this disease occurs in approximately one patient per year per one million individuals. This incidence is uniform across the world. The disease usually comes on in older individuals in the age range of 55-70 years. These individuals have no demonstrable mutations in their prion protein and the mystery is why PrP^{Sc} suddenly appears in the brain and initiates the autocatalytic reaction that leads to recruitment of increased amounts of PrP^{c} and the rapid development of spongiform encephalopathy. It has been postulated that sporadic disease may be the consequence of exposure to prions from other species, but this has not been substantiated. In New Zealand and Australia where the sheep do not carry Scrapie, the incidence of sporadic CJD is also approximately one case per year per one million individuals. Furthermore, recent data suggest that the protease resistant PrP^{Sc} is found in the central nervous system of these sporadic cases but not in the lymphoreticular system. Thus, it is unknown why these individuals suddenly manifest the multimer of PrP^{Sc} **(Fig. 10)** that initiates the onset of spongiform encephalopathy in these older individuals.

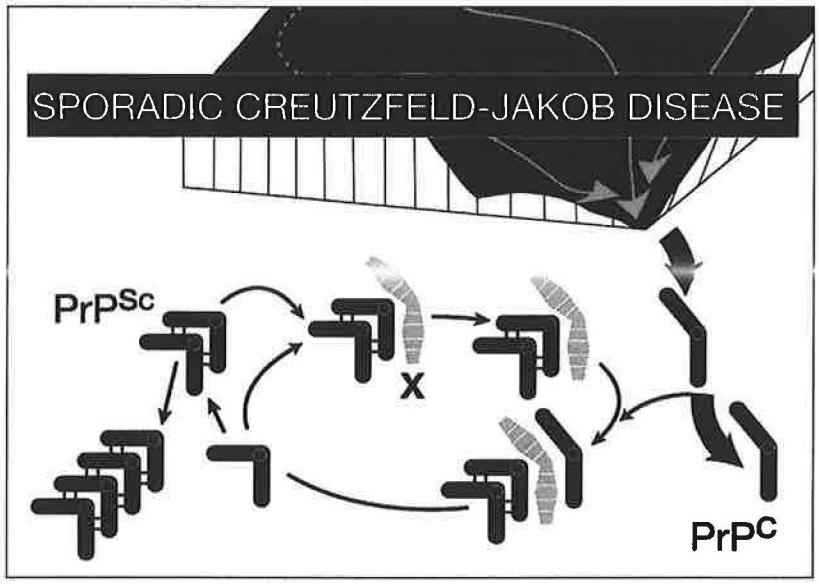


Figure 10

In a third type of spongiform encephalopathy, the individual acquires the infectious PrP^{Sc} multimer from some exogenous source. This infectious particle may enter the body either parenterally through a surgical procedure or injection, or orally through intake in the diet. Furthermore, the offending particle may have come from another human or from a different species. As illustrated in **Fig. 11**, this infectious multimer initiates the autocatalytic reaction that recruits the individual's own PrP^{c} and converts its to the misfolded, polimerized form. This series of events can initiate clinical Creutzfeldt-Jakob disease in the relatively short time of only a few years.

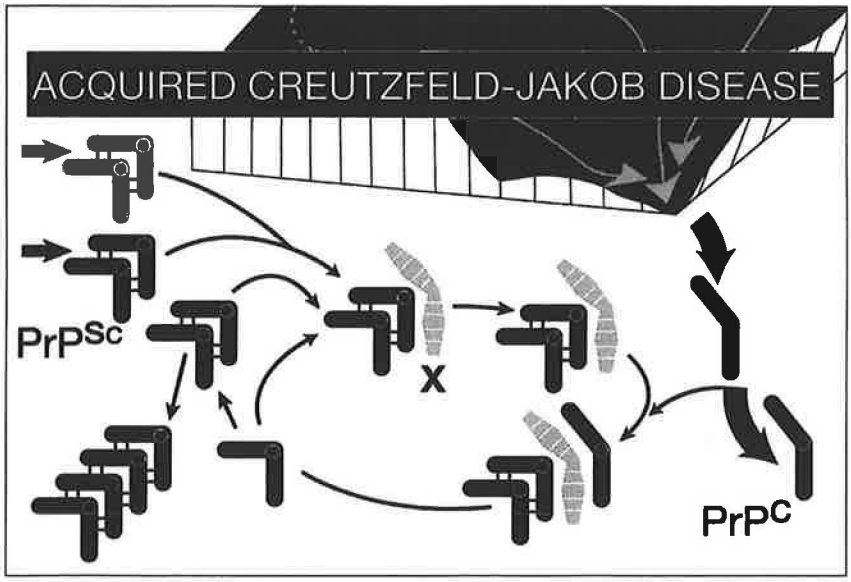


Figure 11

VII. MOVEMENT OF PRIONS FROM THE GASTROINTESTINAL TRACT TO THE CNS

In order to acquire Creutzfeldt-Jakob disease from the environment, it is clear that mechanisms must be available to move the infectious agent from the intestinal lumen into the body, and from the organs of the body to the central nervous system. These pathways have been clarified over the last two years. As outlined in **Fig. 12**, it is now clear that the lymphocyte plays a key role in the transport and reproduction of the infectious prion. Lymphatic tissue expresses the prion protein. In addition, if the lymphatic tissue acquires the infectious multimer, abnormal prion formation can take place within this tissue. In the infected animal or human, for example, it is now possible to demonstrate proliferation of the protease-resistant prion in lymph nodes, the spleen and other lymphoid tissue. It is now thought that lymphocytes may acquire the prion from the diet by actually migrating between the epithelial cells in the jejunum and, particularly, the ileum. These cells then migrate to the large collection of lymphocytes in the terminal ileum, right colon and appendix where prion replication can be demonstrated. The lymphocytes, and particularly the B cells, may then migrate throughout the body to lymph nodes, the spleen and tonsils. In addition, within the germinal centers, the follicular dendritic cells also become infected and these cells, in turn, are thought to play a role in directly transferring the prion to the autonomic nerve terminals that are closely associated with this lymphoid tissue. There is then retrograde transport of the prions into the spinal cord and up into the remainder of the central nervous system. Thus, different tissues will manifest protease-resistant prion deposition depending upon the site of entry of the infected material. If the infected material is inserted directly into the CNS then prions are found only in the brain and not in any of the tissues in the body. If this infected material is injected parenterally (for example, into the peritoneal cavity) then lymph nodes, the spleen, the tonsils and the CNS manifest prion replication while the lymph tissue in the ileum, right colon and appendix remains uninfected. Finally, if the infected material is acquired from the diet, then prion replication can be demonstrated in the lymphoid tissue in the intestine, colon and appendix as well as the lymphatic tissue throughout the rest of the body. Recent experiments have shown that in the absence of B cells or follicular dendritic cells (which depend upon B cells for maturation), the prions cannot be transferred into the CNS.

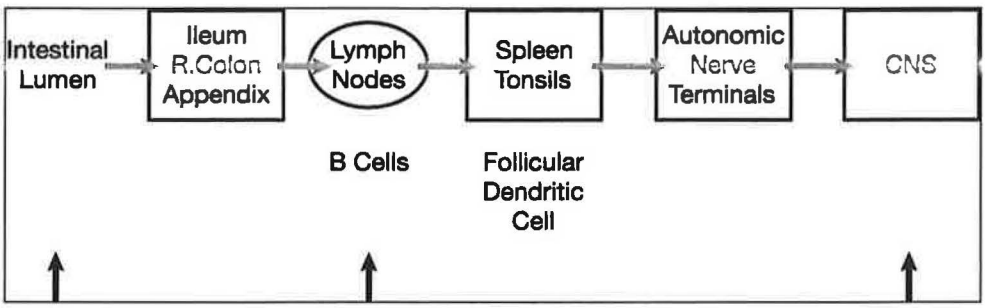


Figure 12

VIII. TISSUES WHICH HARBOR PRIONS AND, THEREFORE, ARE POTENTIALLY INFECTIOUS

From these latter considerations, it is clear that those tissues that are involved in the transport of prions throughout the body may also potentially transmit the disease to other animals who may ingest or be injected with these tissues. This question is particularly important with

respect to the manner in which animals are slaughtered and prepared for human consumption. **Fig. 13** illustrates the potential infectivity of different organs in animals such as sheep, cattle, and deer. Not surprisingly, the tissues of the central nervous system are highly infectious as are major peripheral nerves. The second group of organs, that carry the infectious prions, include those tissues with significant amounts of lymphoid tissues such as the spleen, tonsil, ileum, right colon and lymph nodes. Other major organs, such as the liver, have substantially lesser amounts of the infectious prion and muscle tissue contains very little. However, a very recent study has demonstrated that if a tissue is injured and a chronic inflammatory reaction is introduced into that tissue, there is migration of infected lymphocytes into the region and the tissue then becomes highly infectious. Thus, it is difficult to be certain that meat is free of prions since it may be contaminated during the slaughtering process with parts of the central nervous system, may contain large peripheral nerves or may be infiltrated with contaminated lymphocytes. As will be discussed later, it is also clear that the lymphocytes contained in a blood transfusion can transmit the disease to the recipient.

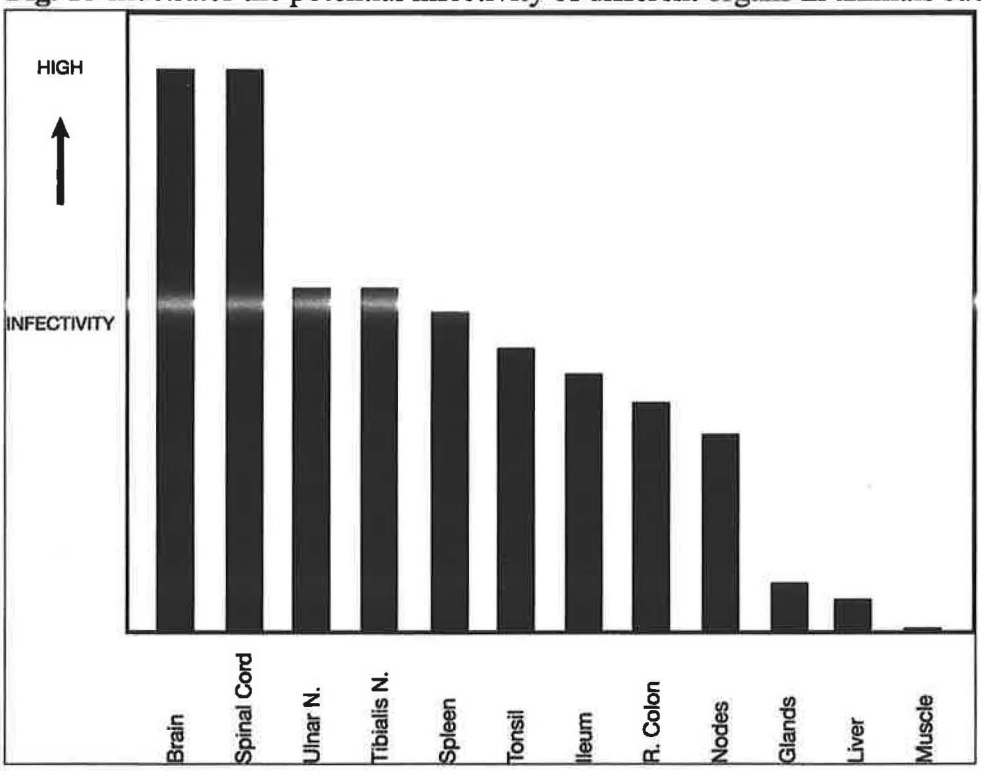


Figure 13

IX. SYNDROMES OF ACQUIRED CREUTZFELDT-JAKOB DISEASE

Over the past 30 years, there have been a number of outbreaks of acquired spongiform encephalopathy that illustrate many of the problems associated with this disease.

Neurosurgical Transmission

There have been a number of reports of patients who developed acquired Creutzfeldt-Jakob disease following a variety of procedures in which instruments or electrodes were inserted into their brain. Presumably, patients with unrecognized Creutzfeldt-Jakob disease were subjected to neurosurgical procedures, biopsy or electrophysiological studies and the instruments used in these procedures became contaminated with prions. Since these prions are highly resistant to the usual sterilization procedures carried out in hospitals, the instruments were infectious and transmitted the disease to the next patients that were instrumented. As an aside, it has been demonstrated that small pieces of stainless steel wire contaminated with prions will

transmit the disease to a recipient even though these pieces of wire have been subjected to a variety of sterilization procedures including intense ultra violent light, formaldehyde treatment, various gas sterilizing techniques, and heating to 100 degrees for 30 minutes. Only exposure of these wires to concentrated sodium hydroxide solutions will render them sterile. Clearly, the problem in these cases was inadvertent contamination of neurosurgical instruments with prions that subsequently were passed on to other patients. The latent period for the development of Creutzfeldt-Jakob disease under these conditions can be very short and of the order of 1.5 years.

Dura Mater Grafts

Another source of prions that can be transmitted directly to patients is dura mater grafts. This material was usually harvested from cadavers and then, unfortunately, was processed in batches for use in subsequent neurosurgical procedures. Nearly all of the reported cases came from a single type of graft, the Lyodura brand of graft processed before May, 1987. While these cases were seen around the world, the majority occurred in Japan. As illustrated in **Fig. 14**, the use of these grafts became fairly common in the late 1970's and throughout the 1980's. The first recognized case of Creutzfeldt-Jakob disease in an American recipient of these grafts was reported in 1987. Over the years, there have been approximately 150 cases of this form of acquired Creutzfeldt-Jakob disease around the world. These findings led to major revisions in the harvesting and processing of these grafts. Much greater attention is paid to the medical history and autopsy findings of the donors, the grafts are processed singly, rather than in batches, and each dura mater graft is treated with 1.0 N sodium hydroxide. The obvious problem here is that there presumably was an unrecognized case of sporadic Creutzfeldt-Jakob disease in one of the donor cadavers from which the dura was harvested. Because of the batch processing, this particular dura contaminated the entire batch. In general, the incubation period between the time of receipt of the graft and the development of the syndrome was approximately eight years although this latency period ranged from 1.3 to 16 years.

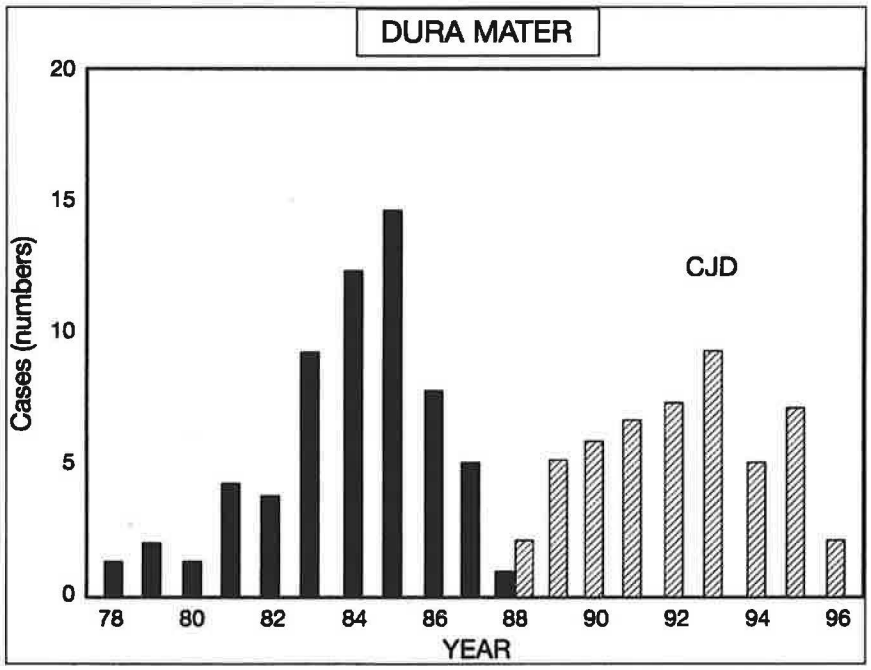


Figure 14

Human Pituitary Hormone Extraction

In the early 1950's a group of children were identified who failed to grow because of a deficiency in pituitary-derived growth hormone. While other hormones such as insulin could be extracted from commercial animals such as sheep and cattle and used in humans, growth hormone from these sources was ineffective in treating these children. Effective growth

hormone had to be recovered from a primate in order to work in the human. As a consequence, a series of "pituitary collection societies" were established in France, Great Britain and the United States. The function of these groups was to collect pituitaries that had been harvested at autopsy. These pituitaries were frozen, collected into batches, and processed for extraction of growth hormone. The batches of pituitaries varied from 500 to 10,000 glands. The treatment of the children with pituitary insufficiency proved to be highly effective and nearly 30,000 children ended up receiving this therapy by 1985. It should be noted that use of this material ceased in 1988 when synthetic human growth hormone became available. Several years after the first injections began, cases of acquired Creutzfeldt-Jakob disease in the recipients began to appear. The proportion of the children treated with this preparation who developed spongiform encephalopathy in five different countries averaged about one case in every 75 children who were treated. This proportion varied greatly from country to country, however, and was one in 300 in the United States, one in 45 in Great Britain, and one in 20 in France. In more recent years, as summarized in **Fig. 15**, the great majority of the cases occurred in France. These differences in attack rates probably reflected differences in the manner in which the hormone was extracted and prepared for injection in the different countries. In the United States the preparative work included a column chromatography step that is now known to have largely separated the growth hormone from contaminating prions. In France, such a chromatographic step was not included in the purification. As of the end of 2002, in the United States there have been no further cases of this disease. These tragic cases provided new information on the transmissibility of the prion. It now seems obvious that in harvesting pituitaries from the general population, a gland from a patient with sporadic Creutzfeldt-Jakob disease was occasionally included in the batches. Since these pituitaries were all processed in large batches, if such a diseased pituitary was included, the entire batch became contaminated. What is novel about the transmission of this disease, however, is that the contaminated material was administered parenterally, and not directly into the CNS as was the case with the two previously described groups of patients. Clearly, some mechanism must exist whereby prions from the body can be transmitted across the blood brain barrier and into the brain.

Country	Number of Deaths
France	89
UK	41
US	25
New Zealand	5
Netherlands	2
Brazil	1
Australia	1
Quatar	1

Figure 15

Kuru

Additional insights into the mechanisms of transmission of the prion particle came from a description of an outbreak of acquired Creutzfeldt-Jakob disease in the Fore tribe of Papua New Guinea. In the 1940's the interior of Papua New Guinea, was densely populated with a number of tribes, some of whom were cannibals. There had been virtually no contact between people from the outside world and these interior tribes. After the end of World War II, the Australians administered Papau New Guinea, and began a campaign to "pacify" the various tribes and to try to eliminate cannibalism. As part of this campaign, social and health workers were sent into the interior to visit various tribes. These groups also included anthropologists and public health officials. In 1953 a young German physician visited the Fore tribe and reported a very unusual disease. Many women and children in the Fore villages were dying of a progressive neurological

disease known as Kuru (the Fore language term meaning "to tremble with fear or cold"). The members of the tribe considered this illness to be due to sorcery and each time a victim would die, some other member of the tribe, or a neighboring tribe, would be killed as the presumed perpetrator of the sorcery (a form of ritual murder called *tukubu*). Thus, each year several hundred members of the community were dying under circumstances where the total population of the tribe was relatively small. Zigas provided the first detailed epidemiological descriptions of the disease. It was very puzzling that Kuru occurred in the Fore people and not in the surrounding tribes. Blood samples that he collected and sent for outside analysis revealed no evidence of inflammation or infection. In 1956 he obtained a brain from an affected individual and this led to the recognition that the individuals with this disease had a spongiform encephalopathy. Curiously, the disease involved only the women and children in the village while the men were seldom affected. Investigations revealed that the unique difference between the Fore people and their neighbors was the performance of a curious funeral ritual carried out at the death of an elder. Following the death of an older member of the family, out of respect the women (accompanied by the children) would butcher the corpse and would remove the brain and various internal organs. This procedure was carried out with crude metal and stone knives and often resulted in abrasions and cuts on the skin. Portions of the brains and viscera were then eaten by the women and children. The men of the village did not participate in this ritual and were not allowed to eat any tissue other than, possibly, muscle tissue. This observation presumably accounted for the major difference in attack rates in women, children and men (**Fig. 16**) observed in the village. In the early 1950's, the Australian efforts to reduce intra tribal wars and cannibalism began to have a significant effect on the incidence of Kuru in the Fore people. Over the next 30 years (**Fig. 16**) the disease slowly disappeared. One of the more recent striking observations, however, is that virtually all of the women who had participated in ritualistic cannibalism in the 1950's and survived into the 1980's and 1990's were found to be heterozygotes at the 129 locus. Thus, Kuru appeared to be a massive example of genetic selection where the survivors were those patients programmed by heterozygocity at the 129 locus to be resistant to the development of spongiform encephalopathy. It is now postulated that a case of spontaneous Creutzfeldt-Jakob disease occurred in a member of the Fore tribe, possibly in the 1930's or 1940's. Because of the practice of ritualistic cannibalism, the infectious prion from this individual was passed to other immediate members of the family and, subsequently, to other family units within the tribe. The fact that the women experienced numerous cuts and abrasions during the butchering process raised the possibility that

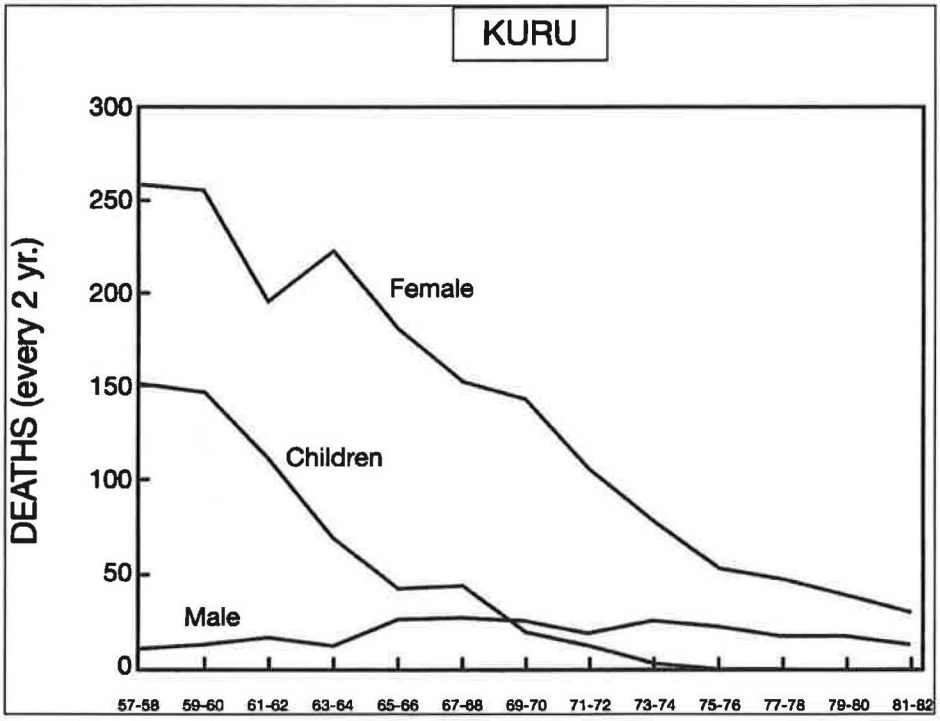


Figure 16

infected brain tissue was being transmitted parenterally. However, the young children that were present during the butchering apparently never had such wounds but were only fed various body parts. This observation, therefore, certainly raised the possibility that the infectious prion particle could be transmitted from the diet, across the gastrointestinal tract, and, ultimately, to the central nervous system. This unsettling possibility was to be confirmed with the outbreak of acquired Creutzfeldt-Jakob disease in the British population in the 1990's.

The British Epidemic

The prion disease Scrapie has been known to be present in sheep in Europe, in general, and in England, in particular, since the mid 1700's. Affected animals began to withdraw from the flock and appeared to have excruciating itching. Despite the fact that they had no objective skin lesions, these animals continuously rubbed and scraped against fence posts, the corner of buildings, trees and other objects until their fleece was torn and their skin abraded. This disease was of concern to the various countries because of the commercial value of the wool fleece. Throughout the 1800's there was extensive epidemiological investigation by veterinarians as to the nature of this disease. It seemed to have a genetic component in that certain strains of sheep were resistant. On the other hand, it seemed to be infectious. The disease might appear in a new flock of animals allowed to graze in the pasture that formerly contained infected animals (like anthrax). In addition, in 1937 it was shown that inoculation of brain tissue from an infected animal directly into the CNS of a normal sheep would result in the production of the disease. In 1938, it was demonstrated that tissue taken from the brain of an infected animal and processed in a way known to inactivate bacteria and viruses would transmit Scrapie to normal animals. Even up to the 1960's it was unclear what the nature of the infectious agent was. Clearly, it had been shown to be resistant to UV radiation, formaldehyde treatment and heat and yet the agent was transmissible by injection directly into the central nervous system of a recipient animal, through parenteral injection and through oral feeding. The infectious agent could clearly cross species boundaries and could easily be transmitted to goats.

In the 20th century, another commercial development was to play a role in the development of the British epidemic. In the early 1900's, it was recognized that growth in young farm animals could be promoted by feeding a meal that was rich in protein and amino acids. Supplementing the diet of newborn animals such as sheep and cows became a common practice throughout Great Britain, as well as other countries in Europe and the United States. For the most part, this meal was the byproduct of the slaughter industry. When animals are slaughtered, meat and any other commercially valuable portions of the carcass are removed. What is left (offal) consists of skin, bone, hoofs, various internal organs, nerves and parts of the central nervous system. All of this residual tissue from slaughtered sheep, hogs and cattle were routinely collected and processed into the food supplement for young animals referred to as "meat bone meal" (MBM). Up until 1980, the processing of this material occurred in large batches that were initially placed in closed ovens and heated for several hours to a high temperature. Excess water was removed from this mixture and excess triacylglycerol was extracted utilizing organic solvents. The final dry, lipid free protein mixtures was ground into a fine powder to be utilized for dietary supplementation in young farm animals. However, in the late 1970's and early 1980's this commercial process was altered. Use of continuous rotating ovens, rather than batch processing, was initiated and a steam extraction process was substituted for solvent extraction. This change was necessitated by federal regulations that were designed to prevent worker exposure to organic solvents. It should be emphasized that because of the huge sheep population in Great Britain, sheep carcasses are a major component of this processed MBM. In addition, it is well established that Scrapie is endemic in at least one third of all flocks of animals in Great Britain.

As illustrated in **Fig. 17.**, in the early 1980's a strange disease suddenly appeared in cattle that was manifest as behavioral and neurological abnormalities that rapidly led to wasting and death. These cases were immediately identified as examples of bovine spongiform encephalopathy. This epidemic rapidly spread (**Fig. 17**) to involve at least 200,000 cows. In general, this disease was never seen in young animals but was usually found in dairy cows that were 5-7 years of age. A link to the feeding of MBM was quickly established and in 1989 a law was enacted prohibiting the feeding of this dietary supplement to young animals. The epidemic in cows rapidly subsided over the following five years. Over the course of this epidemic nearly 200,000 cattle were shown to be infected with prions although it is suspected by many that the number is closer to 1 million animals.

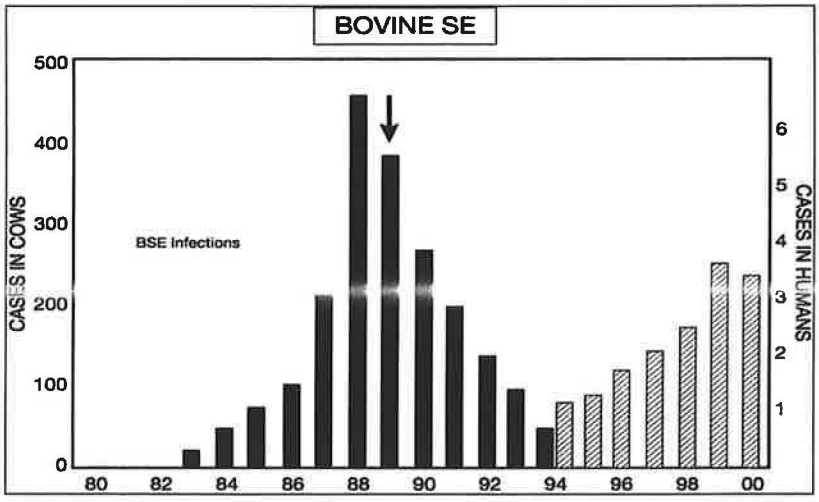


Figure 17

In the early 1990's unusual cases of Creutzfeldt-Jakob disease were beginning to be seen in young humans. In contrast to sporadic Creutzfeldt-Jakob disease, these cases were seen in relatively young people (teenagers and young adults 20-30 years of age) who presented with psychiatric symptoms, depression and withdrawal followed by neurological findings and death within 12-14 months. The number of these cases identified in Great Britain throughout the latter part of the 1990's rapidly increased (**Fig. 17**), To date, the total number of cases of this acquired Creutzfeldt-Jakob disease is approaching 200 (compared to 200,000 cows). Intense investigation has established that the disease in cows represented spongiform Creutzfeldt-Jakob disease caused by the cattle prion and that this infectious agent was transmitted to humans orally and resulted in the initiation of acquired human Creutzfeldt-Jakob disease.

In the last few years, the British government has attempted to define whether or not this epidemic is essentially over. As seen in **Fig. 18**, the number of deaths due to this acquired disease appears to be diminishing. An important aspect of this epidemic is that of the nearly 200 cases investigated, all manifest MM homozygocity at position 129 on the human prion protein. Even though the majority of the population is heterozygous (MV) at this locus, none of the observed cases had this polymorphism. Thus, just as in the Fore people who developed Kuru, the MV heterozygocity as locus 129 apparently prevented the development of CNS disease even when these individuals were exposed to the infectious prion in the diet.

However, within the last year, this concept has taken an unexpected turn, based upon the findings in two recent case reports. One individual was reported who had received a transfusion from a patient who subsequently developed Creutzfeldt-Jakob disease. The recipient of this transfusion developed Creutzfeldt-Jakob disease 6.5 years later. This patient was MM at the 129 locus. This case raised the serious possibility that the prion could be transmitted through blood transfusions. A second similar case was then reported, but in this instance the recipient died of a ruptured aortic aneurism 5 years after receiving the contaminated blood transfusion. This patient was heterozygous at the 129 locus and had no clinical or pathological evidence of prion disease in the CNS. However, his lymphoid tissue showed protease-resistant prion accumulation. This observation, along with studies carried out in the mouse and recently reported in Science, has raised the sinister possibility that heterozygocity at the 129 locus does prevent CNS disease but it does not prevent the proliferation of infectious prions throughout the rest of the body in the lymphoreticular system. These findings raise the possibility that in the English population there is a large, unrecognized population of individuals, MV at the 129 locus, that carry the infectious prion and are potentially capable of contaminating surgical instruments and passing on the disease through blood donations. The British government has now initiated a very large survey, to be completed at the end of 2006, to try to quantitate the seriousness of this potential risk.

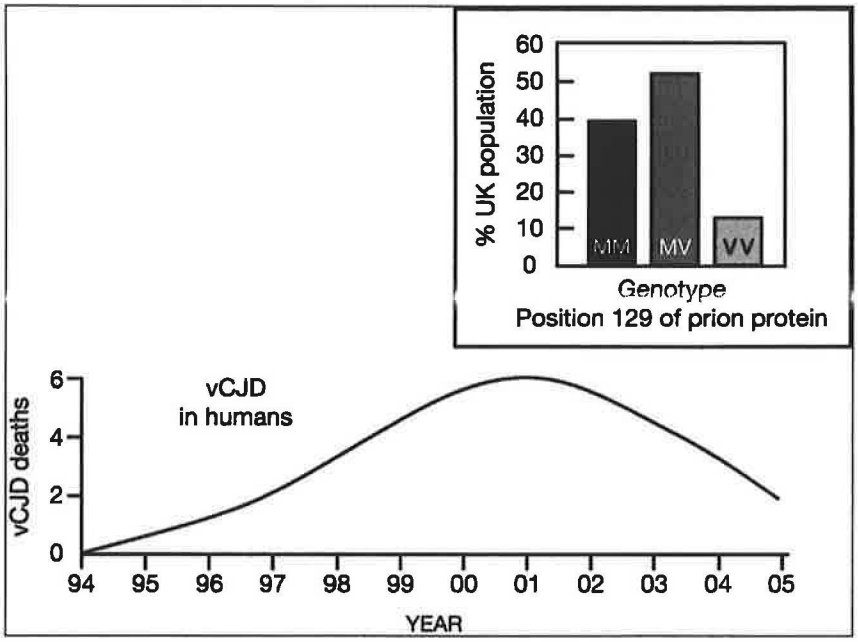


Figure 18

X. PRION DISEASE IN THE UNITED STATES

Generally, the flocks of sheep in the United States were considered to be free of Scrapie. However, the first case in this country was diagnosed in 1947 in a sheep that had been imported through Canada but that had originally come from Great Britain. Unfortunately, the disease has slowly spread to other flocks so that as of 1999 Scrapie has been identified and confirmed in more than 900 separate flocks. Since 1952, there has been in existence a poorly orchestrated control program and, more recently, restrictions have been placed on the interstate movement of sheep from high risk flocks. It would seem that the original animal in this expanding epidemic came from Great Britain where the disease is endemic.

In 1947, the United States also experienced outbreaks of transmissible mink encephalopathy. Additional outbreaks were recorded in 1961, 1963 and 1985. The progress of these outbreaks is being followed by the University of Wisconsin. It is assumed that these outbreaks came from feeding commercial foods that may have been contaminated with sheep products. Thus far, there has been no outbreak in the United States of feline spongiform

encephalopathy, nor has there been an outbreak of this disease in any exotic ruminant animals in the country.

From all of these considerations it is obvious that an outbreak of prion disease could prove to have disastrous consequences for any country. The diagram in **Fig. 19** outlines the worse case scenario that might occur. There are three steps illustrated in this scheme. First, a single animal acquires spongiform encephalopathy, either spontaneously or because of access to contaminated food. Second, if this particular prion is highly contagious it might be rapidly spread to other members of that flock or herd of animals. Third, if this particular prion is capable of crossing the species barrier it might enter the human food chain either directly or indirectly by first being transferred to a domestic animal. It should be noted that in the case of Scrapie, the disease was endemic in the sheep population, it was not particularly contagious and it was transferred into the human food chain only after it became incorporated into meat bone meal.

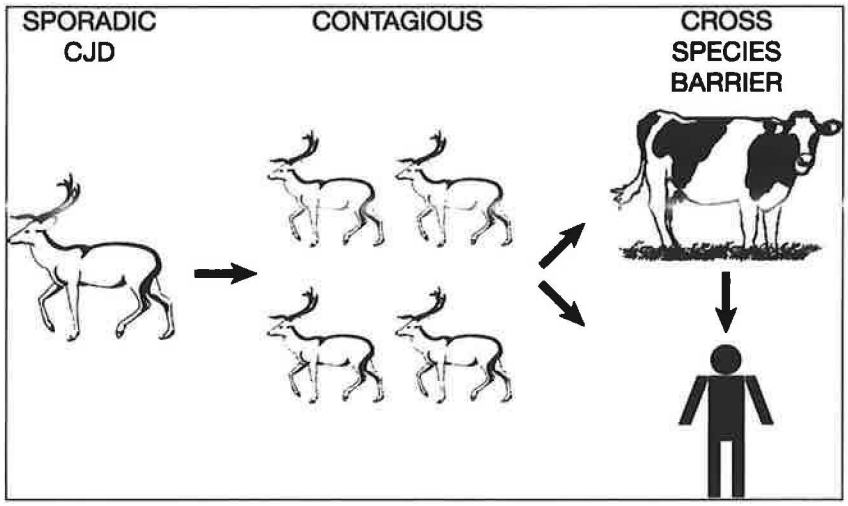


Figure 19

In the United States, a new form of prion disease has appeared. In 1968, a new disease was described in captive deer populations where the animals exhibited abnormal behavior, progressive neurologic dysfunction, an inability to eat and, finally, death. During the ensuing 10 years, this disease, now known as Chronic Wasting Disease (CWD), spread to a number of captive deer populations in northeastern Colorado and southern Wyoming. Only in 1978 was it recognized that CWD was a spongiform encephalopathy. By 1981, it was discovered that free ranging elk had the disease and multiple reports appeared in the next 15 years of free ranging elk and deer that had been harvested by hunters and were found to have the disease.

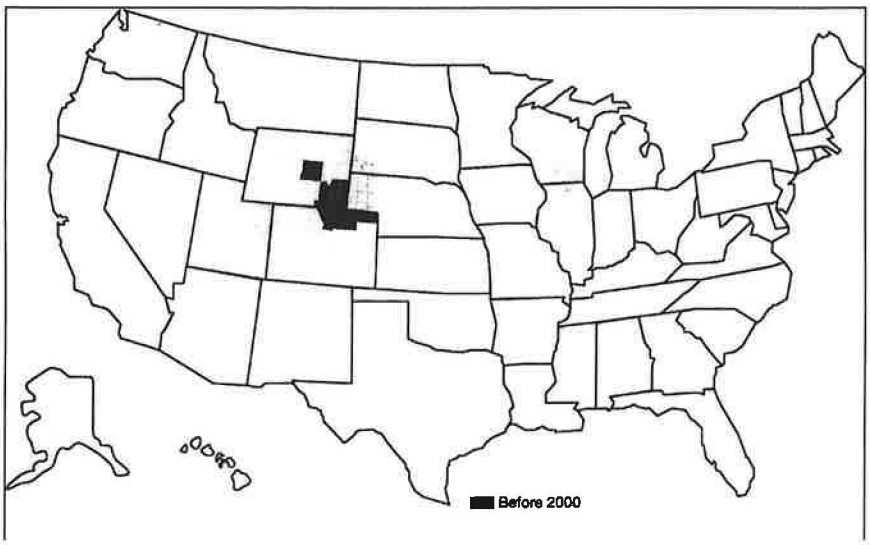


Figure 20

As shown in **Fig. 20**, this disease is spreading with alarming rapidity throughout the country. As indicated by the dark shading, prior to 2000, free ranging elk and deer were identified only in northeastern Colorado, east of the Continental Divide, and in southeastern Wyoming. Since 2000, the disease has crossed to the western slope of the Continental Divide and rapidly spread to most of western Colorado and into Utah. In addition, it has extended up into northwestern

Wyoming and throughout western Nebraska and South Dakota. In 2001, a focus of spongiform encephalopathy was identified in free ranging elk and deer in southern Wisconsin and northern Illinois and, in 2002, a focus of the disease was identified in free ranging deer in far southern New Mexico. The genesis of this disease is uncertain. It may be that the epidemic began in northeastern Colorado because of contaminated food fed to the commercial herds of elk and deer. This, however, has not been established. This disease could be spontaneous. The government has been very lax in controlling this epidemic and in 2002, for example, at least one commercial dealer shipped animals from an infected herd to 14 different states as well as to Canada and South Korea. There apparently were outbreaks of CWD in both Vancouver and South Korea which were rapidly controlled by killing the herds. It is likely that the outbreak in Wisconsin/Illinois came from animals shipped in from Colorado/Wyoming. No point source has been identified for the outbreak in southern New Mexico.

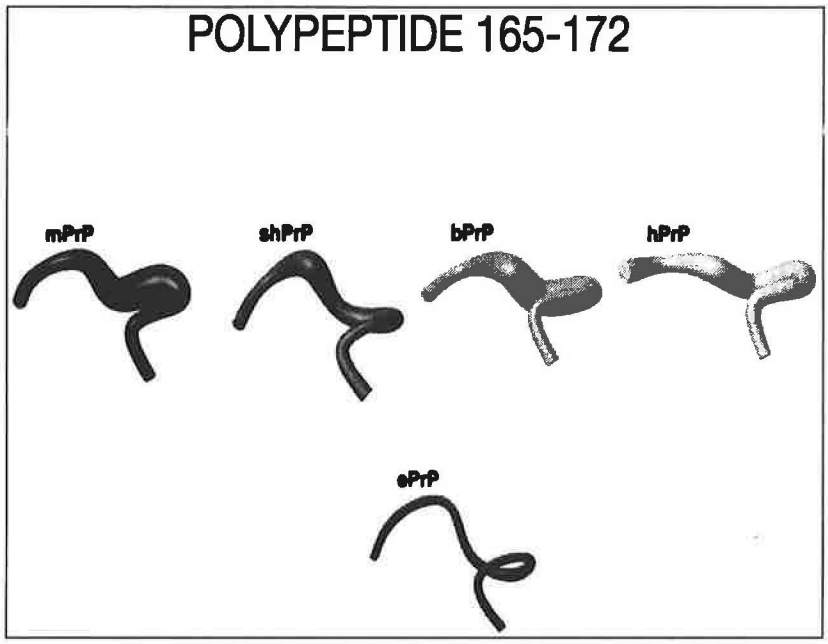


Figure 21

Just three weeks ago in the Proceedings of the National Academy of Science, three papers appeared on the comparative structure of a portion of the prions derived from mice, sheep, cows, humans and elk (**Fig. 21**). The authors of these three papers examined the molecular structure of one portion of the prion protein, i.e., residues 165-172. This portion of the molecule is thought to be critically important for its interaction with the chaperone molecule, protein X, and so is critical for refolding of this protein into the abnormal prion form. In this diagram, the degree of molecular movement in this part of the peptide chain is indicated by the thickness of the line representing this region in the different species. As is apparent, this peptide segment in the elk prion protein (ePrP) is different from this portion of the molecule in all of the other prions. The elk prion protein has two amino acid substitutions that allow this portion of the peptide chain to be tightly hydrogen bonded into the rest of the molecule. The authors suggest that this unusually rigid and well defined structure on the elk prion protein may allow it to act more avidly with protein X and so be more readily converted to the abnormal prion structure. Conceivably, such an arrangement might ultimately lead to greater infectivity among animals.

This possibility has now been tested directly in studies which are ongoing in Colorado and Wyoming. Two herds of animals have been observed for four years. One of these herds represented young animals derived from infected females. The second herd came from females that were not infected with CWD. In both cases, over a four-year period of observation, virtually 100% of the animals developed spongiform encephalopathy. Clearly, this disease is far more infectious than a disease like Scrapie where the current infectivity rate in a flock containing the disease is approximately one new case per 100 animals per year. This infectivity rate is probably 20 times higher in flocks of deer and elk. More worrisome, it is now clear that CWD is spreading widely among free ranging animals. When deer or elk in various test areas in northern Colorado are examined (after being killed by hunters), the incidence of CWD has increased from

around 5% in 1994 to more than 50% in 2000. These data, it should be emphasized, come from the original outbreak area.

A number of studies are also underway to determine if the abnormally folded elk prion is capable of inducing PrP^{Sc} formation from the normal prions isolated from other elk, sheep, cows and humans. As summarized in **Fig. 22**, in this in vitro study, PrP^{Sc} derived from infected elk are incubated in vitro for three days with normal prion protein derived from elk, sheep, cow and humans. As seen in the graph, the abnormal elk prion can readily convert elk PrP^{c} to PrP^{Sc}. Similarly, it can also convert the normal prion protein derived from sheep, cows and humans to PrP^{Sc}, although the efficiency of this conversion process is considerably less in these other species.

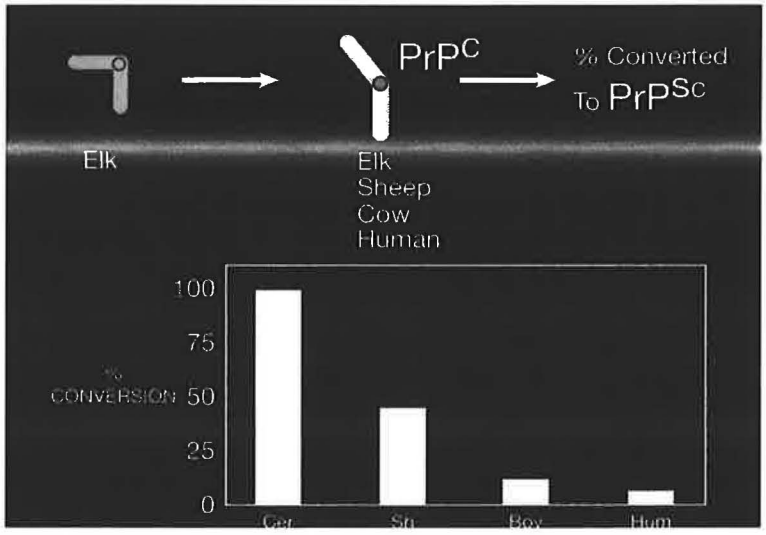


Figure 22

Studies have also been undertaken where infected brain from sick elk have been injected directly into the brain of mice, mink, cows and monkeys. In all four cases, this resulted in the generation of spongiform encephalopathy. In the case of the cows, however, only 3 of 13 animals thus far developed the disease. This is in contrast to 9 cows injected with the sheep prion in which case all 9 animals developed spongiform encephalopathy. There is little doubt that this prion from elk can induce spongiform encephalopathy in a number of other species, including primates, when injected intra cerebrally. Most importantly, studies are underway to determine if oral transmission of this prion is possible. One herd of 11 cows has been fed food contaminated with $ePrP^{Sc}$. Thus far, no cows have developed spongiform encephalopathy, although the period of observation has not yet been sufficiently long. Finally, two small herds of cows are also being maintained with captive herds of infected deer and elk. So far, none of these cows has developed spongiform encephalopathy.

Thus, these very important studies are underway and at least raise the possibility that the elk prion can induce spongiform encephalopathy in other species. Whether this transfer can take place in the wild to mountain sheep or mountain goats or to domestic herds of sheep or cattle remains to be seen. Finally, **Fig. 23** reports a cluster of three young humans recently reported to have died from spongiform encephalopathy in the Wyoming/Colorado area. Two of these patients were 28-30 year old males who were active deer hunters and ate deer meat intermittently throughout the year. One of these individuals was VV and the other was MV at the 129 locus. The third patient was a 28-year-old female whose father was an active deer hunter and whose uncle lived in Wisconsin and hunted elk. The family intermittently ate both types of meat. She was MM at the 129 locus. All three of these young adults developed early mental and cognitive changes and died within a year of progressive neurologic disease.

Where this story goes from here, we do not know.

	Patients		
	1	2	3
Age / Sex	28, Female	30, Male	28, Male
Presentation	Mental and Gait Changes	Cognitive Difficulties	Memory, Behavior Changes
Codon 129	MM	VV	MV

Figure 23

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