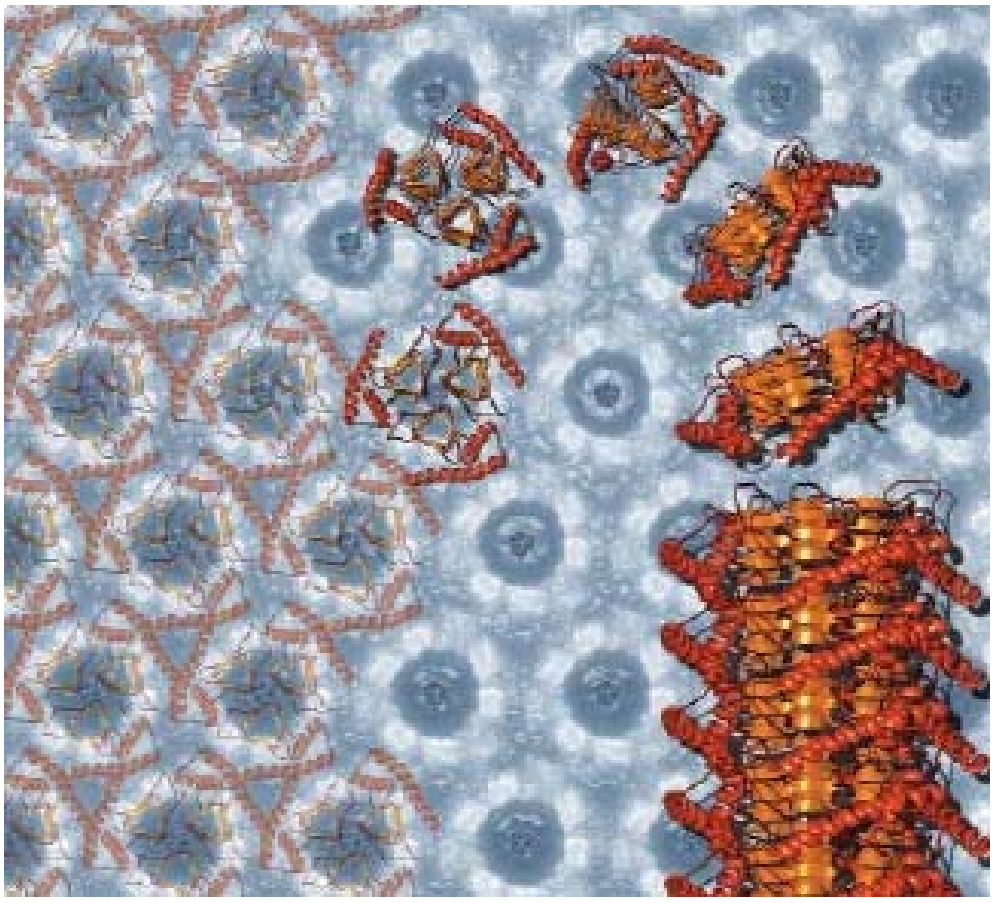
THE CREUTZFELDT-JAKOB DISEASE IN THE LIGHT OF THE PROTEIN-ONLY HYPOTHESIS AND THE CONTRADICTORY MYCOBACTERIUM ASSUMPTION



PRESENTED BY

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Abstract

Transmissible spongiform encephalopathies (TSEs), such as the Creutzfeldt-Jakob disease in humans, are fatal degenerative disorders of the central nervous system (CNS) occurring in a handful different vertebrates. The common pathological pattern is abnormally staining deposits in the CNS known as amyloid plaques.
Amyloid plaques are composed of fibrils built from a series of polypeptide chains layered one over the other as a continuous stack of β-sheets, termed *cross-beta filaments*. These tend to be highly resistant to proteolysis and are found as well in a number of prominent, non-transmissible neurodegenerative diseases as Huntington's, Alzheimer's and Parkinson's.
Although there are different solid hypothesis about the infectious agents responsible for TSEs, the initial cause of these diseases has yet to be proven.

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Introduction

The Creutzfeldt-Jakob disease (CJD) is a chronic neurodegenerative disorder pertaining to the group of transmissible spongiform encephalopathies (TSEs), frequently also called prion diseases.
All TSEs are fatal infectious, neurodegenerative diseases of the central nervous system. These naturally occurring human and animal illnesses include, beside CJD, the Gerstmann-Sträussler-Scheinker disease, Fatal Familial Insomnia and Kuru in humans, Scrapie in sheep and goats, wasting disease in deer and the more recently observed bovine spongiform encephalopathy (BSE) in cattle.
In most cases, these diseases are associated with the accumulation of an abnormal, pathological isoform (PrP^{Sc}) of the host-derived prion protein (PrP^{C}). The conversion from the normal PrP^{C} conformation to the pathological PrP^{Sc} isoform of the same protein is observed as well in all four variations of the CJD. [2][7][10][48][52]

The inherited, sporadic and iatrogenic form of the CJD

Three of the four different types of CJD have already been known since 1920. The worldwide incidence is about 1/1'000'000 per year with especially high rates in Libya, North Africa and Slovakia.
The statistical distribution of the different variations is the following: 10-15% of the cases are due to a genome dependent, inherited variation that shows an especially high occurrence in families carrying a Met/Met (80%) or Val/Val (10%) homozygoty on codon 129 of the PRNP gene coding for the PrP protein.
The biggest part (85-90%), however, is due to a sporadic mutation in the PRNP gene.
Finally, 5% of the cases are due to iatrogenic infections that might arise during the treatment of infertility or panhypopituitarism (deficiency of growth hormone production in the pituitary gland = hypophysis) with hormones from deceased people or during cornea or dura mater (tough outermost membrane covering the brain and spinal cord) transplantation. [5][15][25]

The consequences of the Creutzfeldt-Jakob disease are severe. During the incubation period that might last for decades, no external symptoms are visible or perceivable. However, once the disease breaks out it takes only 4 to 6 month until the patients decease, at an average age of 60 to 65 years.
Primary observed symptoms are paramnesia, sleep- and coordination disorders as well as spasms. Later on, a complete lack of mental presence follows.
The final cause of death can, up to today, only be proven by an autopsy that reveals a spongy degeneration of the brain (Fig. 1) and accumulation of abnormally staining amyloid plaques. These are aggregates of the so-called PrP^{Sc} prion protein. [13][29][31][36]

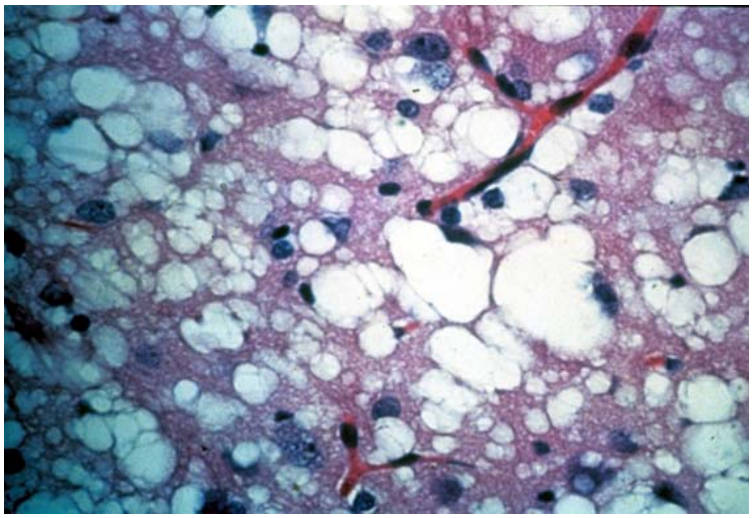

Fig. 1: Spongiform, degenerated brain tissue.

The origin of the nvCJD

The fourth variation of the CJD, the new variant CJD, was described for the first time in 1996 by a British team and is considered a consequence of a trans-species infection with a special protein, called prion. The origin of this nvCJD was found in a TSE of sheep, named Scrapi. This disease has been known for more than 250 years already, especially in England, where the incidence reporting the sheep population is still 0,5-1% a year.
Epidemiological studies revealed two interesting points. First: Human beings cannot be contaminated with Scrapi through the consumption of any part of the sheep. Second: If sheep offals are given to cattle, they might develop a bovine spongiform encephalopathy (BSE). [22][39]

In the United Kingdom the feeding of cattle with sheep offals had been a widespread technique to increase cattle growth rates and meat production for a long time. In the 1980^{s}, however, due to the commercial crisis, processing temperatures were reduced to 80°C, which is not sufficient to denature the infectious agents.
The inactivation of prions, which after the current opinion are probably the infectious particles, is attained only at a temperature superior of 134°C for a minimum of 20minutes. [4][56]
Therefore, in the years after the commercial crisis cattle were fed with potentially infectious animal parts, what in fact resulted in a highly increased number of BSE cases.

BSE on the other hand can cause nvCJD in humans if any of the following parts of an infected cow are eaten: Brain, spinal cord, spleen or intestine. There is no risk of infection with muscle meat unless it has been contaminated during production processes. [11]

The characteristics of the nvCJD

The incubation period lasts, like in the other types of CJD, about a decade. Despite this similarity, the time between the outbreak of the disease and death is about 14 month, which is much longer than in the classical variants. The average age is 29 years, the symptoms are similar to those observed in the other forms of CJD and autopsy shows the same amyloid plaques (Fig. 2). [29][33]

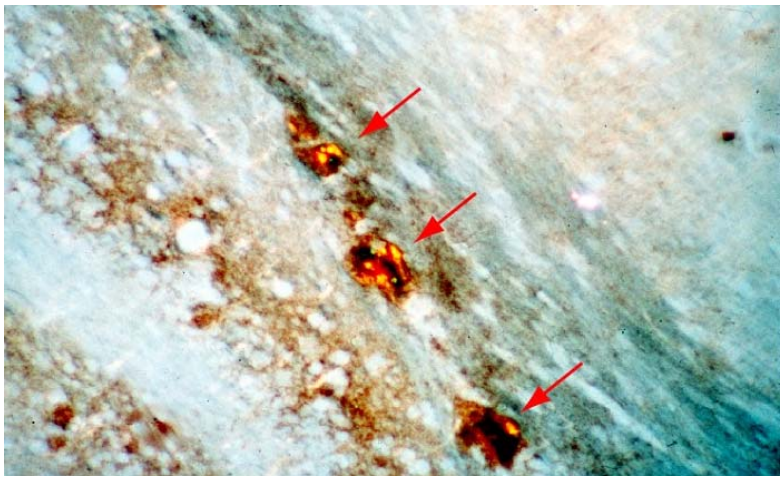

Fig. 2: Stained amyloid depositions (arrows).

Prions, the transmissible agents causing TSEs

The prion hypothesis states that transmissible spongiform encephalopathies are mainly, if not solely, caused by unique transmissible agents consisting of proteinaceous infectious particles, termed prions (**pr**oteinaceaous **i**nfectious **on**ly, Stanley Prusiner 1982).
According to this hypothesis, the key event in the pathogenesis is the conversion of the α-helix rich host prion protein (PrP^{C}) into the pathogenic isoform (PrP^{Sc}) characterized by its insolubility, its high content in β-sheet, and its protease K resistance (Fig. 3 and Fig. 4).

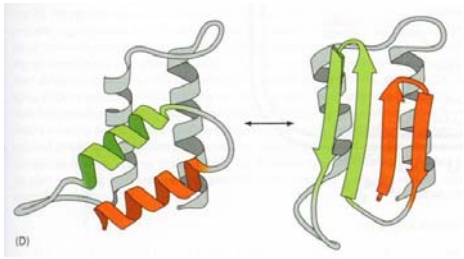

Fig. 3: One of several possible models for the conversion of PrP^{C} to PrP^{Sc}, showing the likely change of two α–helices into four β–strands. Although the structure of the normal protein has been determined accurately, the structure of the infectious form is not yet known with certainty because the aggregation has prevented the use of standard structural techniques. [6]

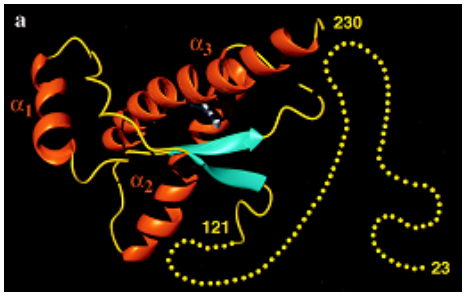

Fig. 4: Diagram of the three-dimensional structure of the intact human prion protein, hPrP (23-230). The α-helices are orange, the β-strands cyan, the segments with non-regular secondary structure within the C-terminal domain yellow, and the flexibly disordered "tail" of residues 23-121 is represented by yellow dots. [43]

The infectious process is believed to follow an autocatalytic mechanism in which PrP^{C} is converted into the misfolded state by PrP^{Sc}. PrP^{C} recruited and misfolded by PrP^{Sc} subsequently catalyses the conversion of further PrP^{C}, eventually leading to massive replication and the formation of *cross-beta filaments* that will form amyloid plaques. [3][35][42][44][45][46][49]

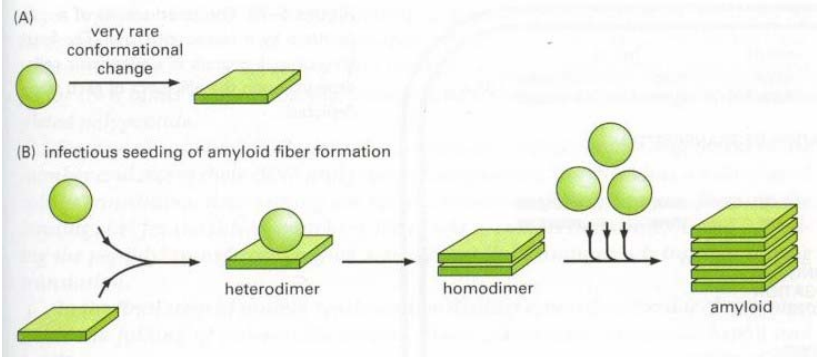

Fig. 5: (A) Schematic illustration of the type of conformational change in a protein that produces material for a cross-beta filament.
(B) Diagram illustrating the self-infectious nature of the protein aggregation that is central to prion diseases. PrP^{C} is highly unusual because the misfolded version of the protein, called PrP^{Sc}, induces the normal PrP^{C} protein it contacts to change its conformation, as shown. Most of the human diseases caused by protein aggregation, e.g. Huntington's and Alzheimer's disease, are caused by the overproduction of a variant protein that is especially prone to aggregation, but because this structure is not infectious in this way, it cannot spread from one animal to another.
(C) Drawing of a cross-beta filament, a common type of protease-resistant protein aggregate found in a variety of human neurological diseases. [6]

Conformation and function of prions

The prion protein PrP^C is a 209 amino acid long glycoprotein located at the cell surface, where it is bound by a glycosylphosphatidylinositol anchor (GPI anchor) (Fig. 6). GPI anchors are a type of lipid linkage by which some membrane proteins are bound to the membrane. They are formed as the proteins travel through the endoplasmic reticulum.
PrP^C is present in a variety of tissues and is mainly expressed on the surface of neurons in the central nervous system as well as on epithelial cells. This specific restriction of expression is probably due to the fact that neurons and epithelial cells are both developmentally derived from the ectoderm, and show an intrinsic polarity of their cell layout.
As all glycoproteins, PrP^C carries oligosaccharide chains covalently linked to amino-acid residues (Oligosaccharides are represented in blue in Fig. 6).

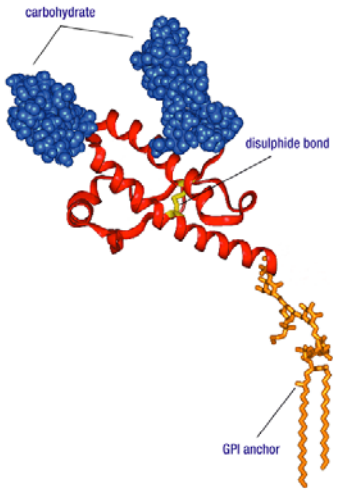

Fig. 6: Model structure of the human PrP^C prion protein. The glycosylphosphatidylinositol anchor (GPI anchor) in orange and the carbohydrates in blue are associated with the protein. However, the PRNP gene that codes for the rest of the PrP^C protein does not code for them.

The N-terminal region of the protein (23-120) is highly flexible and largely unordered whereas the C-terminal region is made up of three α–helices and two short β–strands.

Although the precise physiological function of PrP^C remains to be established, a wealth of experimental data demonstrates its essential role in the susceptibility and pathogenesis of TSEs. For example, removal of the PRNP gene renders mice unable to replicate murine-adapted Scrapie strains, and increasing PrP^C over expression levels in transgenic mice generally reduces the incubation time. [48]

One of biomedicine's longest-running controversy

For years, biologists have tried to prove that a protein called PrP can misfold and become infectious, causing different types of transmissible spongiform encephalopathies. Much effort has been put into the purification of protein clumps from diseased brains, in order to obtain pure filtrates that were subsequently injected into healthy animals. However, it has never been clear that PrP alone was injected. Meanwhile, using synthetic misfolded PrP has not reliably triggered disease.
These two facts might explain why no one has yet accomplished the gold-standard experiment (Koch-Henle's postulates): infecting normal mice, not transgenic ones, with pure prion proteins. [30][34]

Mycobacterium, an old culprit, reappears

Since the prion hypothesis has not yet been proven, many scientists are looking for other possible mechanisms of transmissible spongiform encephalopathies. Recently, Roels and Walravens isolated *Mycobacterium bovis* from the brain of a cow with the clinical and histopathological signs of bovine spongiform encephalopathy (BSE). Moreover, epidemiological maps of BSE incidence in the UK significantly match those of England's bovine tuberculosis distribution (Fig. 7).
At the moment there is no known disease which better fits into the pathological pattern of BSE and other TSEs than bovine tuberculosis, with its blood–brain barrier passing, virus-like forms. [30][34]

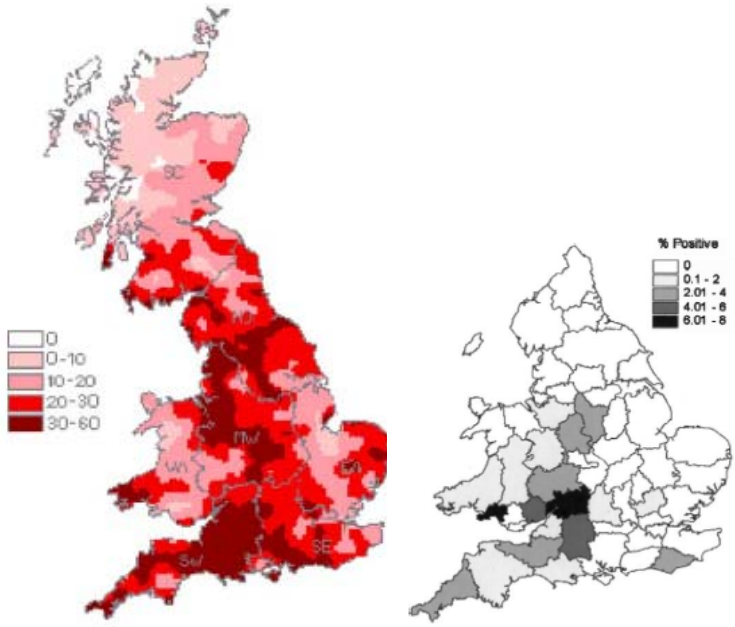

Fig. 7: Distribution of BSE-positive cattle in the UK in 1997 (left) and *Bovine tuberculosis* in England in 1999 (right).

Mycobacterium and the bovine spongiform encephalopathy

By 1975, a new problem had arisen in the wildlife of southwest England: Bovine strains of mycobacterium were isolated from the badger *Meles meles*. Since the bovine form of tuberculosis had been supposedly eradicated since 1960 and mycobacteria were not known to be able to cross species barriers, this development was especially surprising.
On one hand, it was not clear how the badgers of southwest England had acquired bovine tuberculosis and on the other hand, epidemiological studies showed that not a single badger from other areas of the UK harboured the disease.
Other studies showed that tuberculosis, evoked by *Mycobacterium bovis*, can cause the progressive ataxia and the histopathology found in BSE, commonly called mad cow syndrome. Furthermore, *M. bovis* is able to infect all warm-blooded vertebrates and therefore cross species barriers.

Another interesting point is that the protease resistance found in the PrP^{Sc} form of the prion protein is shared as well by the cell-wall deficient forms of *M. bovis* causing bovine tuberculosis. Additionally, the detection of a special 14-3-3 protein in cerebrospinal fluids, originally thought to be a highly reliable indicator for prion diseases, also appears in the central nervous system of tuberculosis victims.
In the same way, the histopathological recognition pattern of TSEs, amyloid plaques, has been implicated as being caused by bovine and human tuberculosis (as well). [30]

Conclusions

The Creutzfeldt-Jakob disease is a rare neurodegenerative disorder belonging to the large group of transmissible spongiform encephalopathies. Their common feature is, as suggested by the name, the spongiform degeneration of the central nervous system observed post mortem as well as the amyloid plaques that appear, however, also in a handful of other diseases.
These diseases, including among others Alzheimer's, Huntington's, Parkinson's and different types of tuberculosis, have a much higher incidence and hence efficient treatment implicates large social and economical consequences. Therefore, research has to be done in order to be able to treat or prevent these illnesses.

The current opinion is that transmissible spongiform encephalopathies are caused by prions. However, it has not yet been possible to prove this notion, and the hypothesis itself fails to explain why other diseases, like Alzheimer's, show the same histopathology.
In addition, there is growing evidence that mycobacteria, known to cause various types of tuberculosis, might be implicated in the infectious process of transmissible spongiform encephalopathies. Nonetheless, further research has to be done in this field, the goal being to be able to explain the formation of amyloid plaques in concerned diseases.

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