Prion diseases, or transmissible spongiform encephalopathies (TSE), are a group of neurodegenerative disorders affecting both humans and animals. Prion diseases are unique in that they can be inherited, can occur sporadically, or be infectious.

Stanley Prusiner introduced the term prion - (proteinaceous infectious particle) and proposed that the infectious agent in prion diseases is composed mainly or entirely of an abnormal conformation of an otherwise normal host-encoded glycoprotein called the prion protein (PrP). This hypothesis was initially greeted with great skepticism in the scientific community but then later was widely acknowledged to be true. Prusiner was awarded the 1998 Noble Prize for Science for his groundbreaking research.

Prion diseases typically have long incubation periods and are rapidly progressive once clinical symptoms begin. They are invariably fatal, with no effective form of treatment. Currently, however, increased understanding of their pathogenesis has led to the promise of effective therapeutic interventions in the near future.

**Etiopathology and Genetics (Table 1)**

Prion protein (PrPc) is a normal protein coded for by a gene - the human PRNP gene (PRNP) located on chromosome 20. PrPc is a glycosylphosphatidylinositol-anchored cell-surface glycoprotein; It may have a role in cell adhesion or signaling processes, but its exact cellular function remains unknown. The N-terminal region of PrP contains a high-affinity binding site for copper ions; hence, PrP may have a role in copper transport or metabolism. PrP is expressed at highest levels in the CNS, in particular in neurons but also expressed widely on cells of the immune system.

A prion disease occurs when the normal protein PrPc exists in an abnormal form - PrPSc (scrapie variant). PrPSc represents a conformationally modified form (rich in beta-sheet) of PrPc (mainly an alpha-helical structure). The high beta-sheet content correlates with PrPSc resistance to enzymatic digestion and infectivity.

The change from PrPc to PrPSc can occur by a spontaneous mutation in the PRNP gene or inheritance of the abnormal gene or when pre-formed PrPSc is introduced into normal healthy tissue which surprisingly results in further conversion of normal PrPc into abnormal PrPSc by a self-perpetuating
vicious cycle. This is followed by abnormal processing of neuronal proteins, diminished clearance and intra-cellular accumulation followed by neuronal death.

Recent research shows that PrPSc is heterogenous with the existence of several distinct isolates or strains which are associated with differing PRNP genotypes and also have a major influence on the disease phenotype in both sporadic and familial human prion diseases.² (see later)

### Pathology

A unifying feature of all the prionoses is their neuropathology. These illnesses tend to affect the gray matter of the central nervous system (CNS), producing neuronal loss, gliosis, and characteristic spongiform change. In addition, plaques with the typical staining properties of amyloid (e.g. apple-green birefringence after Congo Red staining when viewed under polarized light) are observed in many of these conditions. vCJD is characterized by florid plaques (Daisy plaques) throughout the cerebrum and cerebellum.

### Clinical Forms

The most common prion disease is CJD, which occurs world-wide with a uniform incidence of approximately 1 case per million population internationally. The disease is well recognized in India³, but probably underreported. Over the period spanning from 1968-1997, National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore recorded 69 cases of CJD from different parts of India in the CJD registry.

In Libyan-born Israelis and some populations in restricted areas of Slovakia, the incidence of CJD is 60-100 times greater than expected. These local high rates of CJD are linked to a high prevalence of codon 200 mutations in the PRNP gene.

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<tr>
<th>Disease</th>
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² see later

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Table 1: Prion-Related Diseases, Hosts, and Mechanism of Transmission

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CJD

Sporadic CJD (sCJD)
The mean age of onset of sporadic CJD is around 60 years. The incidence of sCJD is about 1 case per million population; however, among individuals aged 60-74 years, the incidence is 5 cases per million population. The age range can be broad; cases have been reported in people as young as 17 years and as old as 83 years. The illness is characterized by rapidly progressive multi-focal neurological dysfunction, myoclonic jerks, a terminal state of global severe cognitive impairment, akinetic mutism and death with a mean duration of around 8 months.

About 40% of patients with sCJD present with rapidly progressive cognitive impairment, 40% with cerebellar dysfunction, and the remaining 20% with a combination of both. The clinical picture rapidly expands to include behavioral abnormalities, cortical visual abnormalities, and both pyramidal and extrapyramidal signs.

Almost all patients with sCJD develop myoclonic jerks that involve either the entire body, face or a limb. These myoclonic jerks can occur spontaneously or can be precipitated by auditory or tactile stimulation with prominent “startle myoclonus”.

The diagnostic criteria for sCJD are:

Definite sporadic CJD
Neuropathological / immunocytochemical confirmation is required for a diagnosis

Probable sporadic CJD
Patients will have rapidly progressive dementia, and at least two of the following four symptoms:

a. myoclonus
b. visual or cerebellar problems
c. pyramidal or extrapyramidal features
d. akinetic mutism

plus typical electroencephalogram (EEG) with generalised triphasic periodic complexes at approximately 1 per second or clinical criteria for possible sporadic CJD (see below) and a positive assay for 14-3-3 protein in the cerebrospinal fluid (CSF) - possible sCJD patients will have a rapidly progressive dementia with two out of the four symptoms (a to d) and a duration of less than 2 years but without positive EEG.

Familial CJD (fCJD)
Ten percent of cases of CJD are familial, with an autosomal dominant pattern of inheritance linked to mutations in the PrP gene. fCJD has an earlier mean age of onset from 45-49 years and mean duration of illness of around 26 months.

Iatrogenic CJD (iCJD)
Many cases of iCJD have been reported though the incidence is decreasing. Transmission has occurred by surgical instruments, EEG electrodes, corneal transplants, dura mater grafts, human pituitary-derived gonadotrophins, and human-derived growth hormone

CJD: Implications of genotype at codon 129 of PRNP

Recent research has confirmed that codon 129 genotype and consequently the PrPSc strain may influence the clinical presentation in CJD and also the susceptibility to CJD.

Atypical forms of sCJD have been well recognized since decades. 10% of cases of CJD have a much more prolonged clinical course with a disease duration of over 2 years. Another 10% may present with
pure cerebellar ataxia rather than cognitive impairment, the so-called ataxic CJD. Heidenhain’s variant of CJD refers to cases in which cortical blindness predominates, with severe involvement of the occipital lobes. The panencephalopathic type of CJD refers to cases with extensive degeneration of the cerebral white matter in addition to spongiform vacuolation of the grey matter, and has been predominantly reported from Japan. Amyotrophic variants of CJD have been described with prominent early muscle wasting.

In humans the methionine (M) / valine (V) polymorphism at codon 129 of PRNP is associated with different PrPSc types and based on this, sCJD has been divided into six subtypes: sCJDMM1/sCJDVM1, sCJDVV2, sCJDMM2, sCJDVV1, and sporadic fatal insomnia (sFI). Over 70% of cases of sCJD have the MM genotype with a statistically significant shorter duration of illness. Codon 129 heterozygosity increases the duration of illness. To date, variant CJD has occurred in only the MM genotype. MM cases show typical EEG readings in >50%. This contrasts with VV cases which are all atypical for this investigation.

Bovine Spongiform Encephalopathy (BSE) and variant CJD (vCJD)

BSE originated in the United Kingdom in the 1980s, probably from cattle feed that was initially contaminated with tissue from sheep infected with Scrapie. Remnants of slaughtered cattle that had fed on the infected material were then used again as a source for feed, resulting in the continual recycling of material from BSE-infected cattle and infection of large numbers of cattle.

Extensive research suggests that vCJD in humans is caused by ingestion of beef products contaminated with BSE. vCJD has been limited to Europe, with almost all cases occurring in the United Kingdom. Till December 2003, 143 cases of vCJD have been diagnosed in Great Britain with 137 deaths. vCJD has also been reported from France and other European nations all areas where BSE has been reported. Primary cases of vCJD have not been seen in India or other nations where BSE has not been reported.

The clinical findings in cases of vCJD differ dramatically from those in sporadic cases. The symptoms develop at a mean age of 26 years - nearly four decades earlier than in patients with sporadic disease. Clinically psychiatric abnormalities and sensory symptoms are much more common at presentation - many patients present with prominent neuropsychiatric symptoms including irritability, anxiety, apathy, insomnia, and social withdrawal. The neuropsychological profile is one of a combined cortical and subcortical dementia, with impaired executive function, faces recognition and visuoperceptual function. Patients generally show fluctuations in the efficiency of their performance with prominent distractibility, intrusion and perseverative errors.

The diagnosis may not be suspected until the neurologic symptoms appear, including cognitive impairment, pain and paresthesias, dysarthria, and gait abnormalities. Myoclonus is a late feature, and startle myoclonus is rarely elicited. Cerebellar findings on the other hand are present in all patients with established vCJD, while only about 40% of those with sporadic CJD have cerebellar dysfunction.

vCJD has a slightly longer course, with a mean duration of 16 months to death and differs from all other human prion diseases in that the disease-associated form of the prion protein and infectivity are present in lymphoid tissues throughout the body.

The diagnostic criteria for vCJD are:

Definite vCJD

Patients will have a progressive neuropsychiatric disorder and neuropathological confirmation of the disease, showing spongiform change and extensive PrPSc deposition with orid plaques throughout the cerebrum and cerebellum.
Probable vCJD
Patients can be classified under two sets of criteria:

I. Progressive neuropsychiatric disorder of a duration greater than 6 months where routine investigations do not suggest an alternative diagnosis. And at least four of the following five symptoms:
(a) early psychiatric symptoms (depression, anxiety, apathy withdrawal, delusions)
(b) persistent painful sensory symptoms (including both frank pain and/or unpleasant dysaesthesia)
(c) ataxia
(d) myoclonus or chorea or dystonia
(e) dementia
EEG will not show the typical appearances of sporadic CJD, MRI brain -symmetrical high signal in the posterior thalamus.
No history of potential iatrogenic exposure.

II. Alternatively progressive neuropsychiatric disorder for a period of longer than six months, where routine investigations do not support an alternative diagnosis, and where there is no history of potential of iatrogenic exposure, plus a positive tonsil biopsy which is positive for PrP-res.

Other Prion Diseases

Scrapie
This was the first prion disease to be described - a disease of sheep recognized for over 250 years. The illness, manifested by hyperexcitability and ataxia, leads to paralysis and death. It is called scrapie because of the tendency of affected animals to rub against the fences of their pens in order to stay upright, reflecting their cerebellar dysfunction.

Kuru
Kuru was an illness linked to ritualistic cannibalism among the Fore people living in the highlands of New Guinea, which has now virtually disappeared with the end of cannibalistic rituals. The illness was probably initialized by the consumption of an initial patient with sporadic CJD. Kuru was once the major cause of death among Fore women; because it was part of the ritual cannibalism for women to eat the brains of the dead (neural tissue having the highest dose of PrPSc).
Patients clinically presented with difficulty walking and developed progressive signs of cerebellar dysfunction. Death occurred approximately 1 year following onset of symptoms. Gajdusek who elucidated the transmissibility of kuru was awarded the Noble Prize in 1976.

Gerstmann-Sträussler-Scheinker disease (GSS)
This is an autosomal dominant syndrome caused by mutations of the PRNP gene. Patients with this illness present with a slowly progressive limb and truncal ataxia, with dementia. Death occurs 3-8 years following presentation.
The prominent involvement of the brainstem often leads to symptoms suggestive of olivopontocerebellar degeneration. The neuropathology of GSS is remarkable in that extensive and invariable amyloid deposition occurs, in addition to the typical spongiform change.

Fatal familial insomnia (FFI)
All patients with FFI have a missense mutation at codon 178 of the PrP gene. Patients with FFI present with intractable insomnia, dysautonomia (i.e. hyperthermia, hypertension, tachycardia, tachypnea, hyperhydrosis), dementia, and motor paralysis; however, the phenotypic
expression is very variable even within the same family. The age of onset is also variable, ranging from 18-60 years. Once symptoms begin, the course ranges from 6 months to 3 years. Because of the diversity of clinical presentations of this disorder, genotyping is very important for definitive diagnosis.

Neuropathologically, marked atrophy of the anterior ventral and mediodorsal thalamic nuclei occurs because of neuronal loss and gliosis.

Chronic Wasting Disease (CWD) of Mule/Deer/Elk

Recently, a number of cases of apparent sporadic CJD have occurred in the United States among young individuals (<30 y). Over the same period, a major outbreak of CWD occurred among the deer and elk populations in many western states\(^6\).

The pathology of CWD has many similarities to BSE, including the presence of florid plaques. Significantly, transmission studies of CWD -PrPSc in the laboratory have shown that it can cross the species barrier from deer to human PrP at about the same efficiency as the BSE prion agent. These observations have led to the speculation that limited transmission of CWD to humans has already occurred in the United States.

Investigations in Prion Disorders

EEG (Fig. 1)

During the course of sporadic CJD, most patients develop a characteristic finding on EEG with generalised bi- or triphasic periodic (1-2 / sec) or pseudoperiodic paroxysms of sharp waves or spikes on a slow background. In some cases, the EEG changes may be initially unilateral. These periodic complexes have a sensitivity and specificity of 67% and 87%, respectively, on a single EEG\(^7\). However, if repeated recordings (possibly weekly) are obtained, more than 90% of patients show periodic EEG abnormalities.
Other conditions in which similar generalised periodic complexes may occur
Metabolic encephalopathy
Certain toxic encephalopathies (e.g. Lithium)
Anoxic encephalopathy
Progressive multifocal leucoencephalopathy
Lewy body disease
Multiple cerebral abscesses
Alzheimer’s disease (rarely)
In vCJD, EEG does not show the typical changes observed in sporadic CJD, and findings often are normal.

CSF
Routine CSF is typically normal in sCJD, although the CSF protein may be elevated slightly (but never >100 mg/dL). Analysis of CSF for certain brain specific proteins, particularly 14-3-3, may be useful in the diagnosis. In an appropriate clinical context, a positive test is strongly supportive of a diagnosis of sCJD and a negative test is unusual.
However 14-3-3 is a normal neuronal protein and is released into the CSF in response to a variety of neuronal insults. It is therefore not valid for discriminating between CJD and other dementias in unselected patients and should not be used as a general screening test for sCJD.
Other illnesses, which can give a positive 14-3-3 test include:
Herpes simplex encephalitis and other viral encephalitides.
Recent cerebral infarction or haemorrhage.
Subarachnoid haemorrhage.
Hypoxic brain damage.
Glioblastoma
Carcinomatous meningitis.
Paraneoplastic encephalopathy.

Imaging (Fig. 2)
Brain MRI may show hyperintense signals in the form of ribbon-like areas of hyperintensity in the cerebral cortex, earliest on diffusion-weighted images.

Fig. 2: Diffusion Weighted MRI showing Abnormal Bilateral Anterior Basal Ganglia Signals
Early changes are also often noted in the anterior basal ganglia and the thalamus on diffusion weighted images and then later on the T2-weighted images.

Two characteristic MRI signs have been described - the “hockey stick” sign, which refers to increased signal in the putamen and head of the caudate nucleus resembling a hockey stick, and the “pulvinar” sign, which corresponds to an unusually bilaterally increased signal in the pulvinar thalamic nuclei. The latter sign has been found especially in patients with vCJD but has also recently been seen in sCJD (so may not be as specific as reported before.)

Tissue Biopsy / Brain Biopsy (Fig. 3)
The need for brain tissue to confirm the diagnosis of sCJD has been a major impediment to definitive diagnosis before death.

However a recent report has shown that PrPSc is deposited in the neuroepithelium of the olfactory mucosa in patients with sCJD, indicating that olfactory biopsy may provide diagnostic information in living patients.

What is intriguing is that this finding may represent that either the olfactory pathway is the outer frontier of a centrifugal prion propagation (that starts in central brain structures) or even be an initial route of infection! Either way this area could serve as a means of spreading prions.

vCJD shows involvement of the lymphoreticular system (lymph nodes, spleen, tonsil and appendix). Tonsil biopsy has been used as a supportive diagnostic test in variant CJD. Its main role is probably to provide support for the diagnosis in cases who have negative MR scans or who have atypical clinical features.

Genetic Analysis
In fCJD, the definitive test is the analysis of the PRNP gene for relevant mutations performed on a simple blood sample. Although a family history is usually present in cases of fCJD, sometimes it is not. Aside from analysis for mutations, genetic analysis allows for the determination of the codon 129 genotype (MM, VV or MV). This may have potential relevance in the full characterization of a case of CJD.
Ancillary Investigations

In cases where the diagnosis is not unequivocal it is important to rule out other causes of dementia, particularly rapidly progressive dementia that could be treatable, such as herpes encephalitis. In herpes encephalitis, the CSF has pleocytosis and an elevated protein content, while in CJD the cell count and protein content are within the reference range. In addition, herpes encephalitis usually has characteristic MRI and EEG findings and CSF-PCR test may be diagnostic.

Other entities that are in the differential diagnosis are neurodegenerative disease such as Alzheimer disease, Pick disease, corticobasal ganglionic degeneration, familial myoclonic dementia, toxic dementias and multisystem atrophy. However, the progression of the dementia and other neurological symptoms is usually slower in all these disorders compared to CJD. The clinical symptomatology of Hashimoto encephalitis can be very similar to possible CJD, with the presence of a rapidly progressive dementia, myoclonus, ataxia, and psychosis; however, this autoimmune disorder responds well to treatment with corticosteroids. Patients with Hashimoto encephalitis do not show the 14-3-3 protein in the CSF nor do they have the typical periodic sharp wave complexes on EEG.

Treatment

All prion diseases are fatal; no effective treatment is available. Clinical studies are currently in progress to evaluate compounds that may inhibit the conversion of PrPc to its pathologic isoforms.

Studies in transgenic mice suggest that some forms of PrPc may resist conformational conversion into pathologic isoforms. Over expression of these “dominant negative” prion proteins may prevent or dramatically retard the development of scrapie in mice.

A number of medications have been shown in experimental systems to be effective at preventing development of scrapie in animals. These have included Congo red anthracyclines, amphotericin B, sulfated polyanions, acridine and phenothiazine derivatives (such as quinacrine and chlorpromazine)

Pentosan polysulphate (poly-b-xylose-2, 3-disulphonate) is a large polysulphonated polyglycoside which slowed the accumulation of prions in sheep infected with Scrapie. It has been suggested that pentosan binds to heparan binding sites on infectious prion proteins, potentially inhibiting further prion production. The drug has to be given directly into the brain because it is unable to cross the blood brain barrier.

Beta-sheet breaker peptides - which interact with the PrPSc structure and break beta-sheets have recently been designed and have shown encouraging results in vitro.

Prevention

The prion agent is remarkably resistant to inactivation; hence, routine sterilization procedures, such as autoclaving, are ineffective. Effective protocols to inactivate the agent exist, which involve the use of sodium hydroxide and/or steam autoclaving at 134 Celsius for 1 hour.

Prion disease has not been reported to spread by blood transfusion or through blood products. However, vCJD is of special concern though currently, no methods exist by which blood can be screened for potential contamination with nvCJD.

Vaccination with recombinant mouse prion protein (recPrP) delays the onset of prion disease in mice and preliminary data suggests that humoral immunity is critical for a therapeutic response. Antibody binding to PrPc and/or PrPSc may possibly interfere with PrPSc-mediated conversion of PrPc to PrPSc.

References