Prions and prion diseases

- prions are novel transmissible pathogens causing a group of invariably fatal neurodegenerative diseases
- can present as genetic, infectious, or sporadic disorders
- all are believed to involve modifications of the prion protein, PrP
- prion: proteinaceous infectious
- incidence of all human prion diseases: 1 in 1,000,000

Prion diseases

human: Creutzfeldt-Jakob disease (CJD)
Gerstmann-Straussler-Scheinker disease (GSS)
fatal familial insomnia (FFI)
fatal sporadic insomnia (FSI)
kuru

animals: scrapie (goats and sheep)
bovine spongiform encephalopathy (BSE or MadCow)
chronic wasting disease (deer and elk)

TSE: transmissible spongiform encephalopathy
Historical background

- in 1920s, Creutzfeldt and Jakob described first cases of progressive mental, motor and neurological deficits in young patients - the eponym 'Creutzfeldt-Jakob disease' (CJD) was first used to describe degenerative CNS diseases

- in 1950s, disease known as 'kuru', in Papua New Guinea discovered; neuropathological similarity between kuru, CJD, and scrapie noted

- in 1960s, the term transmissible spongiform encephalopathy (TSE) applied after discovery of the transmissible ability of both kuru and CJD diseases to chimpanzees

- in 1976, Gajdusek awarded Nobel prize for his work on 'slow virus' infections theory

- in 1980s, the 'protein-only' hypothesis was developed by Prusiner; he was awarded Nobel Prize in Medicine in 1997 for his work on prions; theory remains controversial

- in 1990s, outbreak of BSE in cattle in UK raised public concern and spawned vigorous research efforts to understand mechanism underlying prion diseases

acquired prion diseases in humans include iatrogenic (iCJD) and kuru

- iCJD arises from accidental exposure to human prions through medical or surgical

- kuru arises from participation in cannibalistic feasts

in the late 1950s, an epidemic arose amongst the Fore linguistic group and neighbouring tribes in the Eastern Highlands of Papua New Guinea - investigators realized that kuru was transmitted by the cannibalistic ritual of eating the brains of dead relatives

*epidemic was thought to have originated due to a case of sporadic CJD; this person was subsequently eaten and passed along the prions to relatives (a switch from sporadic to acquired)*
- sporadic CJD (sCJD) occurs in all countries with a random case distribution

- about 15% of human prion diseases are inherited; all involve coding mutations in the prion protein gene (PRNP) and have an autosomal dominant inheritance pattern *(the formation of PrP\textsuperscript{Sc} represents a gain of dysfunction)*

*discovery of inherited prion disorders lends support to prion hypothesis and broaden the clinical spectrum of these diseases to include atypical dementia and fatal insomnias*

* no pathogenic mutations in PrP are found in sporadic or acquired prion diseases

- a common PrP polymorphism at residue 129 is a key determinant of genetic susceptibility to acquired and sporadic prion diseases

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- variant CJD (vCJD) appeared in the UK in 1995 and found to be caused by the same prion strain that causes BSE in cattle

* raised the possibility that a major epidemic of vCJD would occur in the UK as a result of dietary exposure to BSE prions

the patient was an avid eater of meat pies which were made of predominantly animal by-products such as organs (these tissues can contain high amounts of prions)
Incidence of MadCow disease in the UK

* the spread of MadCow was contained by the systematic slaughter of suspected cattle

Incidence of Kuru in Papua New Guinea

* the incidence of kuru was decreased after missionaries in the region convinced the affected communities to stop their cannibalistic ritual

Clinical features of prion diseases

- all the disorders are associated with variable dementia due to loss of specific neurons in the brain (large vacuoles can form in neurons that may fuse to create a spongiform change, hence the term spongiform encephalopathies)

- diseases are characterized by loss of motor control, progressive dementia, paralysis and wasting; in the terminal stage, patient is usually mute, rigid and unresponsive (akinetic mutism)

- CJD has cerebral involvement so dementia is more common; patient seldom survives a year

- GSS is distinct from CJD characterized by cerebellar ataxia and concomitant motor problems, dementia is less common (disease course lasts several years before ultimate death)

- fatal insomnias present with an untreatable insomnia and dysautonomia; pathological changes are characterized by severe selective atrophy of the thalamus

prognosis: death within one year after the onset of symptoms in 90% (further 5% of patients die within the next year)
Potential mechanism of neuroinvasion of prions

Prions: a new form of biological information

- the infectivity of prions is believed to occur solely through the unique conformation of the prion protein (PrPSc); this infectivity is inactivated by agents that denature or hydrolyze proteins such as high concentrations of trypsin or SDS
- procedures that alter nucleic acids such as UV irradiation do not affect infectivity
- in contrast to viruses with a nucleic acid genome that encode information in genes, prions encipher infective properties in the tertiary structure of PrPSc
- PrPSc acts as a template upon which normal PrP is refolded into a nascent PrPSc molecule with the assistance of another protein
**general features of prion proteins**

- resistance to protease treatment (proteinase K)

- insensitive to irradiation such as UV 254nm (suggests that nucleic acids are not present)

- forms aggregates or prion rods

*how are prions different from viruses?*

- prions can exist in multiple molecular forms whereas viruses exist in a single form with a distinct ultrastructural morphology (prion infectivity detected in prion particles with a wide range of sizes)

- prions are nonimmunogenic because the normal proteinPrP<sup>C</sup> renders the host tolerant to PrP<sup>Sc</sup>, viral proteins are seen as "foreign" and almost always elicit an immune response

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**Structure of the PrP protein**

![Structure of the PrP protein](image)
properties of PrP isoforms

- normal form of the protein, PrP\textsuperscript{C}, is a 35kDa cell surface glycoprotein that is anchored to specific areas of the membrane (caveolae or lipid rafts) by a glycosyl phosphatidylinositol (GPI) lipid linker - it may have a role in cell adhesion or signaling processes

- half-life on the cell surface is 5 h, after which protein is internalized by a caveolae-dependent mechanism (conversion to PrP\textsuperscript{Sc} likely occurs during this internalization)

- the acidic pH of the endosomal compartment may facilitate the conformational change

- normal PrP is soluble in detergents and readily digested by proteases

PrP gene structure and expression

- entire open reading frame (ORF) of all known mammalian PrP genes resides within a single exon (this argued against the possibility that alternate splicing of the gene resulted in expression of the disease-causing form of PrP)

- PrP mRNA is constitutively expressed in brain; highest levels of the protein are found in the central nervous system although protein is found in most tissues, especially cells of the immune system
Prion protein isoforms

*western blot analysis*

lanes 1 and 2 - normal
lanes 3 and 4 - scrapie form
lanes 2 and 4 - proteinase K treated

**Formation of PrP^{Sc}**

- process occurs posttranslationally
- PrP^{C} containing a predominantly α-helical structure is refolded to a protein (PrP^{Sc}) with high β-sheet content

PrP^{C}: contains 40% α-helix and little β-sheet
PrP^{Sc}: contains 30% α-helix and 45% β-sheet

*this idea goes against the longstanding belief that the amino acid sequence specifies one biologically active conformation of a protein*

the accumulation of PrP^{Sc} is thought to begin with formation of a “seed”; seed formation is slow which accounts for the delay in clinical onset
Structure features of PrP and PrP\textsuperscript{Sc}

C

important for transmission of prions

D

important in defining the species barrier

E

proposed structure of PrP\textsuperscript{Sc}

A

i

GNNQQNY microcrystal

ii

HET-s amyloid fibril

iii

Sup35, Ure2p, or Rnq1 amyloid fibrils

B

i

PrP\textsuperscript{Sc} \(\beta\)-helical model

ii

PrP\textsuperscript{Sc} \(\beta\)-spiral model

iii

Parallel and in-register \(\beta\)-structure for rPrP amyloic
How do inherited mutations in PrP lead to disease?

- mutations that cause inherited prion disease may produce disease by destabilizing PrP\textsuperscript{C} and predisposing the molecule to aggregation
- mutation might also facilitate the interaction between PrP\textsuperscript{C} and PrP\textsuperscript{Sc} or affect the binding of a ligand or coprotein

Normal function of PrP: copper and zinc homeostasis

- PrP binds to Cu(II) and under some conditions can facilitate its folding into a more protease resistant form
- compounds that chelate copper may be useful in affecting infectivity
- binding of copper and zinc are required for endocytosis of PrP
- PrP may be involved in the transport of zinc or act as zinc sensor
- some believe that upon copper binding, PrP acquires antioxidant ability; loss of this function may lead to induction of apoptosis due to oxidative stress

*disturbances in Cu(II) homeostasis leading to dysfunction of the CNS are well documented in humans (Wilson’s and Menkes disease)*
PrP function and prion disease: mouse models

- transgenic mice expressing different types of prions can be infected with different strains of PrPSc and the development of disease studied

- mice lacking PrP by gene knockout (Prnp0/0) showed no gross phenotype; they were completely resistant to prion disease following inoculation (they failed to replicate prions)

- these mice were shown to have abnormalities in synaptic physiology and circadian rhythms and sleep

- mice overexpressing PrPc have increased rates of PrPSc formation and shorter incubation times until the onset of disease

Prion Strains

- major problem for the “protein-only” hypothesis has been how to explain the existence of multiple isolates, or strains, of prions

- different strains are distinguished by their biological properties; they produce distinct incubation periods and patterns of neuropathological targeting (so-called lesion profiles)

- conventionally, distinct strains of a pathogen are explained by differences in DNA sequence (i.e. HIV)

- some postulate that strain characteristics can be encoded by small nucleic acids, or “coprions” (this coprion, however, should be sensitive to UV, no tests to prove this have been done)
- support for the idea that strain specificity may be encoded by PrP itself came from study of two distinct strains of mink prions that can be transmitted to hamsters

hyper (HY) and drowsy (DY): accumulated PrPSc have different physiochemical properties

- strain-specific migration patterns of PrPSc were observed on gels following limited proteolysis; corresponded to different N-terminal ends and implied distinct conformations

same situation observed in two different inherited human prion diseases

- in FFI, protease-resistant fragment after deglycosylation is 19 kDa whereas fCJD (E200K) is 21 kDa; difference is due to unique sites of proteolytic cleavage at the amino termini (again implies different tertiary structure)

Species Barrier

- transmission of prion diseases between different mammalian species is restricted by a “species barrier”

- on primary passage of prions from species A to species B, not all inoculated animals of species B develop disease; those that do have much longer incubation periods

- on second passage of infectivity to species B animals, transmission parameters resemble within-species transmission
Different PrPC sequences (differentiated by color) dictate the spectrum of allowable PrPSc conformations (depicted as different shapes), and these conformations represent different prion strains.

Infection of species X with a specific prion strain derived from the same species results in faithful propagation of strain characteristics.

Infectivity is associated with conformational properties of a particular prion strain.

Passaging of the same prion strain from species X to species Y and Z (which express nonhomologous PrPC) may have different outcomes. If the PrPSc conformation of the donor strain from species X is not accessible to PrPC of the host species, a barrier to transmission is observed as illustrated for species Y. On the other hand, if the conformation of the donor strain is accessible to the host PrPC, transmission occurs, resulting in emergence of a new strain of prion in the host species (as illustrated by species Z).

Factors that contribute to the species barrier...

- the differences in PrP sequences between prion donor and recipient (for mouse and hamster, the barrier appears to be mediated by only 5 residues that differ in the midportion of the molecule, a.a. 94-188)

- the strain of prion
prion-like behavior of two yeast proteins: support for the prion hypothesis

overexpression of two yeast genes, *URE2* and *SUP35*, lead to a prion-like phenotype ([*URE3*] and [*PSI*])

induction of these prion phenotypes was directly shown to be caused by excess of the proteins, not an excess of *URE2* and *SUP35* DNA or mRNA

the induction effect could also be obtained when only a portion of the proteins (prion domains) was overexpressed, these domains were also important for prion propagation

Ure2 and Sup35 are normally soluble proteins; they form insoluble, proteinase K-resistant aggregates upon conversion to the prion state

Therapeutic approaches for prion disorders

- various compounds, some of which bind to PrP\textsuperscript{Sc}, including polyene antibiotics, dextran sulfate, and \(\beta\)-sheet breaker peptides have been shown to have limited effects in animal models of prion disease

- most show a significant effect only if administered long before clinical onset, and in some cases, with the inoculum

- any ligand that selectively stabilizes the PrP\textsubscript{C} state will prevent its rearrangement and block prion replication (or the production of any toxic intermediate forms of PrP on the pathway to PrP\textsuperscript{Sc} formation)