How Now Mad Cow?

Introduction to Prion Disease and Function

SHP – Neurobiology of Development and Disease
TSE: Transmissible spongiform encephalopathies

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<tr>
<th>Disease</th>
<th>Host</th>
<th>Mechanism</th>
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<td>Human</td>
<td>Cannibalism</td>
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<tr>
<td>Sporadic CJD</td>
<td>Human</td>
<td>Spontaneous PrP(^C) to PrP(^Sc) conversion or somatic mutation</td>
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<tr>
<td>Iatrogenic CJD</td>
<td>Human</td>
<td>Infection from prion-containing material, eg, dura mater, electrode</td>
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<tr>
<td>Familial CJD</td>
<td>Human</td>
<td>Mutations in the PrP gene</td>
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<tr>
<td>nvCJD</td>
<td>Human</td>
<td>Infection from BSE</td>
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<tr>
<td>GSS</td>
<td>Human</td>
<td>Mutations in the PrP gene</td>
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<tr>
<td>FFI</td>
<td>Human</td>
<td>D178N mutation in the PrP gene, with M129 polymorphism</td>
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<tr>
<td>Sporadic fatal insomnia</td>
<td>Human</td>
<td>Spontaneous PrP(^C) to PrP(^Sc) conversion or somatic mutation</td>
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<tr>
<td>Scrapie</td>
<td>Sheep</td>
<td>Infection in susceptible sheep</td>
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<td>Cattle</td>
<td>Infection from contaminated food</td>
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<tr>
<td>TME</td>
<td>Mink</td>
<td>Infection from sheep or cattle in food</td>
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<tr>
<td>CWD</td>
<td>Mule, deer, elk</td>
<td>Unclear</td>
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<tr>
<td>Feline spongiform encephalopathy</td>
<td>Cats</td>
<td>Infection from contaminated food</td>
</tr>
<tr>
<td>Exotic ungulate encephalopathy</td>
<td>Nyala, oryx, kudu</td>
<td>Infection from contaminated food</td>
</tr>
</tbody>
</table>

Pathophysiology Characteristics:

- Brain vacuolation
- Astrogliosis
- Neuronal apoptosis
- Accumulation of misfolded prion plaques.

http://www.emedicine.com/neuro/topic662.htm
Histological Analysis
Scrapie

• Invariably fatal, chronic neurodegenerative disease.
• First reported in England, France and Germany in the 19th century.
• Long period of incubation (2-5 years).
• Affected animals rub their coats against trees, suffer ataxia, convulsions, blindness, anorexia, and eventually death.
• Death usually occurs within 1-6 months.
Kuru

• Discovered by Carleton Gajdusek in the 1950’s and 60’s among the South Fore people of New Guinea.

• Transmitted through ritual mortuary cannibalism where deceased individuals were consumed by their relatives to honor them.

• Shirley Lindenbaum reported that maternal kin would remove the arms and feet of the corpse, strip the muscles and remove the internal organs, including the brain.

• Between 1957 and 1968, over 1,100 South Fore succumbed to kuru. Early on it affected mostly women (80% vs men) but later also affected elderly and children at high rates as well.
Symptoms of Kuru

1) **Ambulatory Stage** – myoclonus, unsteadiness of stance/gait/hands/eyes, dysarthria, slurring of speech, tremor, uncoordination of lower extremities that progresses upwards.

3) **Sedentary Stage** – victim can no longer walk, severe tremors, ataxia, shock-like muscle jerks, emotional lability, inappropriate laughter and extreme depression, cognitive decline.

5) **Terminal Stage** – intensifying symptoms above, urinary and fecal incontinence, dysphagia (difficulty swallowing), lapse into coma, and lose control of breathing.

These symptoms are primary cerebellar in nature and are highly typical of all TSE related pathology.
Early experiments

- Transmissibility was unintentially demonstrated by inoculation of a Scottish sheep herd with a vaccine extract prepared from formalin treated brain of a scrapie-infected animal.
- Within 2 yrs, 10% of the flock contracted scrapie.
- Gajdusek notes similarity in brain pathology between Kuru and scrapie. He goes on to inject chimpanzees with Kuru brain extracts, after which they exhibit TSE pathology.
- Investigators follow up by showing transmissibility to animals of CJD, familial TSE, and GSS.
Paradigm Shift

- In 1967, Alper and his group report the extreme resistance of scrapie infectivity to UV light and ionizing radiation.
- They previously isolate this activity to 200kD, eliminating the role of even viruses as the vehicle.
- In 1967, JS Griffith proposes three possibilities for these findings:
  1) agent is a protein that turns on its own transcription
  2) agent is a variant protein form that can corrupt the native form of protein to its state via oligomerization.
  3) agent is an antibody that stimulates its own production.
Animal Rendering

The practice of processing animal byproducts into commercial material as animal feed.

In September 1995, reporter Van Smith of Baltimore’s Weekly City Paper visited Valley Proteins Inc, a Baltimore rendering plant:

Smith observed these items listed: a horse, the grill grease and used frying oil from Camden yards, a baby elephant who died in Baltimore, Illinois, tons of waste meat and inedible animal parts from the local supermarkets and slaughterhouses, carcasses from the zoo, thousands of dogs, cats, raccoons, possums, deer, foxes, snakes, and the rest of the local animal shelters waste and road kill that must be disposed each month.
Progression of BSE

• 1986: First case of BSE discovered in a cow that was fed livestock feed produced from a sheep that died of scrapie.
• Dr. Richard Lacey announces that scrapie and BSE are the same disease and that “this beef was in the meat supply”.
• British government dismisses Lacey and cuts his research funding. They announce that scrapie renderings are still an acceptable form of livestock feed.
Progression of BSE (cont)

- 1987: 700 BSE infected cows are reported in Great Britain.
- 1988: 7,000 infected cows. Law is passed declaring sheep rendering illegal.
- 1992: 36,000 infected livestock reported.
- 1994: 150,000 infected livestock reported and is identified in half of British cattle herds.
Crossing the line…..

• In 1996, a new form of CJD is discovered in the UK, termed variant CJD (vCJD).
• Linked with consumption of BSE-contaminated beef.
• Shares the symptoms of classic CJD, except the median age of death is 28 (contrasting with 68) and feature psychiatric and sensory symptoms with neurologic effects occurring later.
The purification of the infective scrapie agent revealed a protease-resistant fragment that copurifies with infectivity.

Cloning identifies the gene as 33-35kD glycoprotein PrP.

PrP is insoluble and protease-resistant only in infected animals and accumulates in plaques in infected brain.

Finally, knockout of PrP in mice renders them immune to the effects of infective prion.
<table>
<thead>
<tr>
<th>Species</th>
<th>Start</th>
<th>Length</th>
</tr>
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<tbody>
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<tr>
<td>Bos</td>
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<td>Ovis</td>
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<td>Mus</td>
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<tr>
<td>Homo</td>
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<tr>
<td>Danio</td>
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<td>Danio</td>
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<td>226</td>
</tr>
</tbody>
</table>

Sequence alignments for different species.
Mechanism of Prion Propagation

**STEP 1:** Initial generation of an infectious particle
- Spontaneous
- Inherited

**STEP 2:** Growth by addition to aggregate
- Neuronal toxicity

**STEP 3:** Amplification of infectious particles
- Accumulation of PrP^Sc
- Phenotypic conversion

**STEP 4:** Reinfection
- Oral ingestion
- Cerebral inoculation

**Mammals**
- Spontaneous
- Inherited

**Fungi**
- Spontaneous
- Overexpression of Sup35p
- Nonsense suppression
- Depletion of Sup35p

Cytoduction
- Cytoduction
- Cytoplasmonic during mitosis
Secondary routes of transmission (iatrogenic CJD)

- Dura and corneal transplants.
- Being operated on with surgical tools used on a CJD patient.
- EEG depth probes contaminated by previous patients.
- Blood transfusions?
Protein can convert between two conformations (a benign form and pathogenic state) at a certain frequency.

The second state can seed the formation of oligometric, insoluble aggregates that in turn form toxic amyloid plaques.

During the oligomerization the prions corrupt the native form of the protein into a transmissible disease conformation.

Molecular Mechanism
Molecular prion characteristics

• Usually rich in polar amino acids such as glutamine or asparagine.
• Computational structure prediction suggests poor secondary structure preference.
• The domain is dispensable for the function of the protein.
• The protein can exist in soluble or aggregated form.
definition of amyloid:
filamentous
protease-resistant
high in beta sheet
yellow-green birefringence with Congo Red

Amyloid filaments of the
Ure2 prion domain (Ure2p^{1-65})

Protease K
digestion
0 1 2 5 10 15 min

Ure2p
7-10 kDa

Circular dichroism

Structural Changes Occur in PrP
PrP^c
PrP^{sc}

EM of filaments

Wickner et al, 2004
Yeast prions

- Non-Mendelian genetic element that is transmitted by cytoplasmic mixing.
- Prion phenotype can be reversed by denaturation and arises again spontaneously at low frequency.
- Expression of endogenous gene is required to propagate prion form.
- Overexpression of gene increased spontaneous conversion to the prion form.
- Protein can exist in two states (like PrP): soluble/protease-sensitive and insoluble/protease-resistant.
- Conversion process can be reconstructed in vitro by conversion of native conformation to the prion form by progressive dilution.
- Prion domain is modular and can be transferred to other genes.
Reporter fusion becomes inactivated, and is heritable and dominant

Si, Lindquist, and Kandel, 2003
Prion form is active form
“the good news is that people may not be contracting Alzheimer’s as often as we may think, the bad news is that they may be getting something worse instead.”

-Peter Jennings. ABC World New Tonight
5/12/1997