Viral Encephalitis & Prions

Microbiology Lecture 4 PNS Module

Ashraf Khasawneh
Faculty of Medicine
The Hashemite University

A 24-year-old male college student presents to the emergency department with a 3-day history of fever, severe headache, and progressive confusion. His roommate reports that he has been increasingly disoriented and has had several episodes of vomiting. The patient also complained of photophobia and neck stiffness. On examination, he has a temperature of 102°F (38.9°C), a heart rate of 110 beats per minute, and a blood pressure of 120/80 mmHg. He is disoriented to time and place. Nuchal rigidity is present, and there is no focal neurological deficit. A lumbar puncture is performed. CSF analysis: Opening pressure: Elevated, White blood cell count: 250 cells/µL (predominantly lymphocytes), Protein: Elevated, Glucose: Normal, CSF Gram stain: No organisms seen.

Which of the following is the most likely causative organism?

A. Herpes Simplex Virus (HSV)

B. Neisseria meningitidis

C. Streptococcus pneumoniae

D. Listeria monocytogenes

E. Cryptococcus neoformans

VIRAL INFECTIONS IN THE CNS

Aseptic meningitis

– inflammation of meninges with sterile CSF

Encephalitis

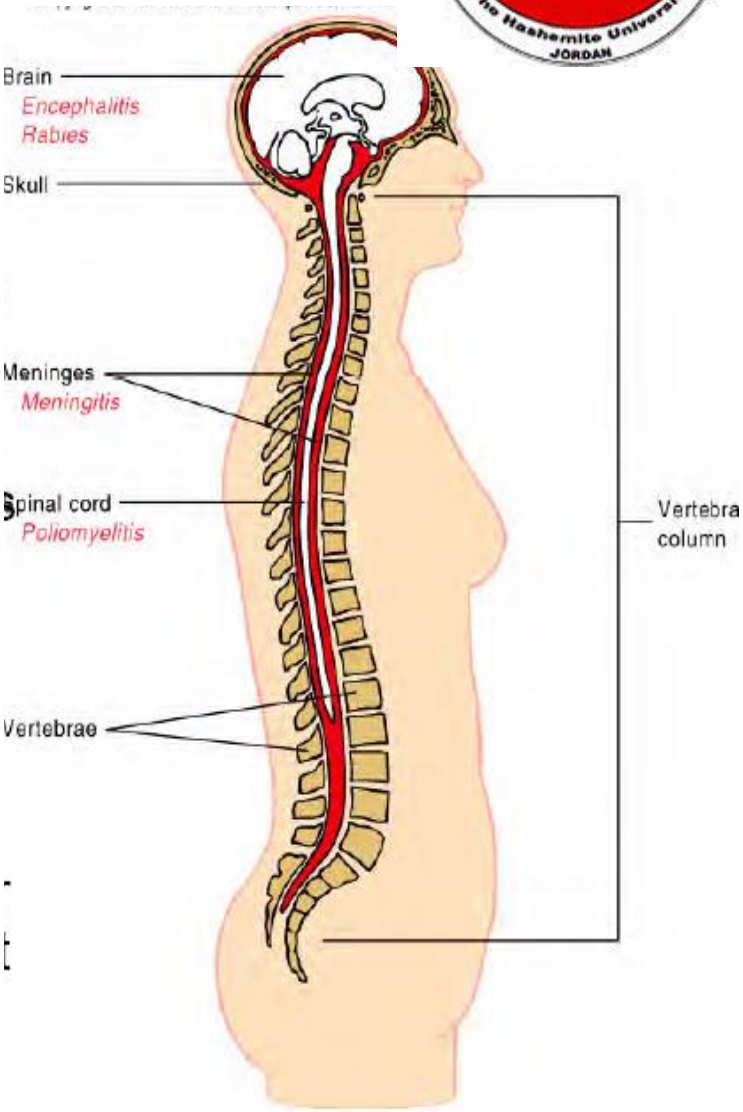
– infection of the brain parenchyma

Meningoencephalitis

– inflammation of brain + meninges

Myelitis

– inflammation of the spinal cord



What is encephalitis?

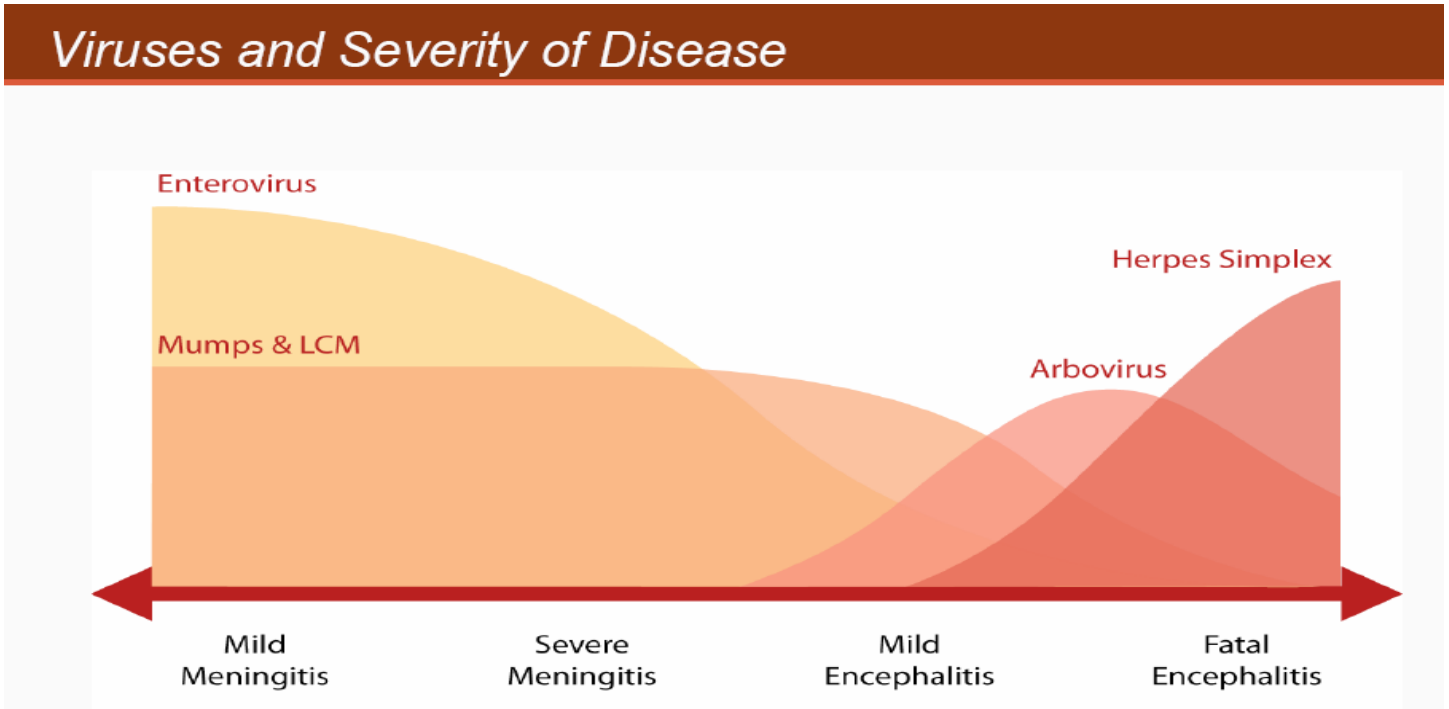
- Encephalitis is an inflammation of the brain tissue due to infection.
- Most often caused by viruses that pass the blood stream into the CSF leading to destruction of neural cells and inflammation of brain parenchyma.
  - Primary or acute encephalitis
- May also result from a viral-mediated inflammatory response in the brain following an acute, systemic infection.
  - Secondary or post-infectious encephalitis

Secondary or post infectious encephalitis

- Subacute sclerosing panencephalitis (defective strains of hypermutated measles virus)
  - CNS involvement (encephalitis) due to cytotoxic (CD8) T-cells which react with virus infected cells.
  - SSPE (1 in 100,000) chronic measles virus infection to CNS. SSPE: personality change, intellectual deterioration, myoclonus, spasticity, tremor and ocular abnormalities
  - Occur 2-10 yrs after infection. No treatment
- Progressive postrubella panencephalitis
  - Mimics SSPE, very very rare, 6 months – 4 yrs
  - Associated with either persistent rubella virus infection of the CNS or late sequalae of congenital rubella infection which manifests in adults.
- Progressive multifocal encephalopathy (polyomavirus JC)
  - Subacute degenerative disease of the brain found in:
    - Immunosuppressive disease: AIDS and hematologic malignancies
    - Disease requiring immunosuppressive therapy
  - No specific treatment, 50% Mortality
- Persistent Enterovirus infection
  - Seen in patients with congenital or acquired immunodeficiency where they develop chronic CNS infection
  - Headache, confusion, lethargy, seizure and CSF pleocytosis.
  - Temporary improvement with type specific immunoglobulins, relapse on withdrawal

How to distinguish encephalitis from viral meningitis?

- Unfortunately, the clinical syndromes and results of routine laboratory tests are typically nonspecific and often do not help distinguish encephalitis and viral meningitis.
- Patients may have symptoms of both parenchymal and meningeal processes.
  - i.e., A patient with stiff neck and photophobia, though classic signs of meningitis, could in fact also have encephalitis! (called Meningoencephalitis)



Encephalitis vs. meningitis

Constitutional symptoms	Encephalitis	Viral Meningitis
Fever	**Yes**	**Yes**
Headache, nausea, vomiting, lethargy	**Yes**	**Yes**
Photophobia, neck stiffness	**No**	**Yes**
Neurologic dysfunction		
Seizures	**Yes**	**Minimal**
Cranial nerve palsies, paralysis	**Yes**	**No**
Altered mental status (i.e. confusion, coma)	**Yes**	**Minimal**

VIRAL MENINGITIS / ENCEPHALITIS

HERPESVIRIDAE
- Herpes simplex
- Varicella-zoster
- Epstein Barr
- Cytomegalovirus

PARAMYXOVIRIDAE
- parainfluenzae
- Mumps
- Measles

MISCELLANEOUS
- Adenoviridae
- Rhabdoviridae
- Retroviridae (HIV)

ENTEROVIRUS
- **Polioviruses**
- **Coxsackie viruses**
- **Echoviruses**

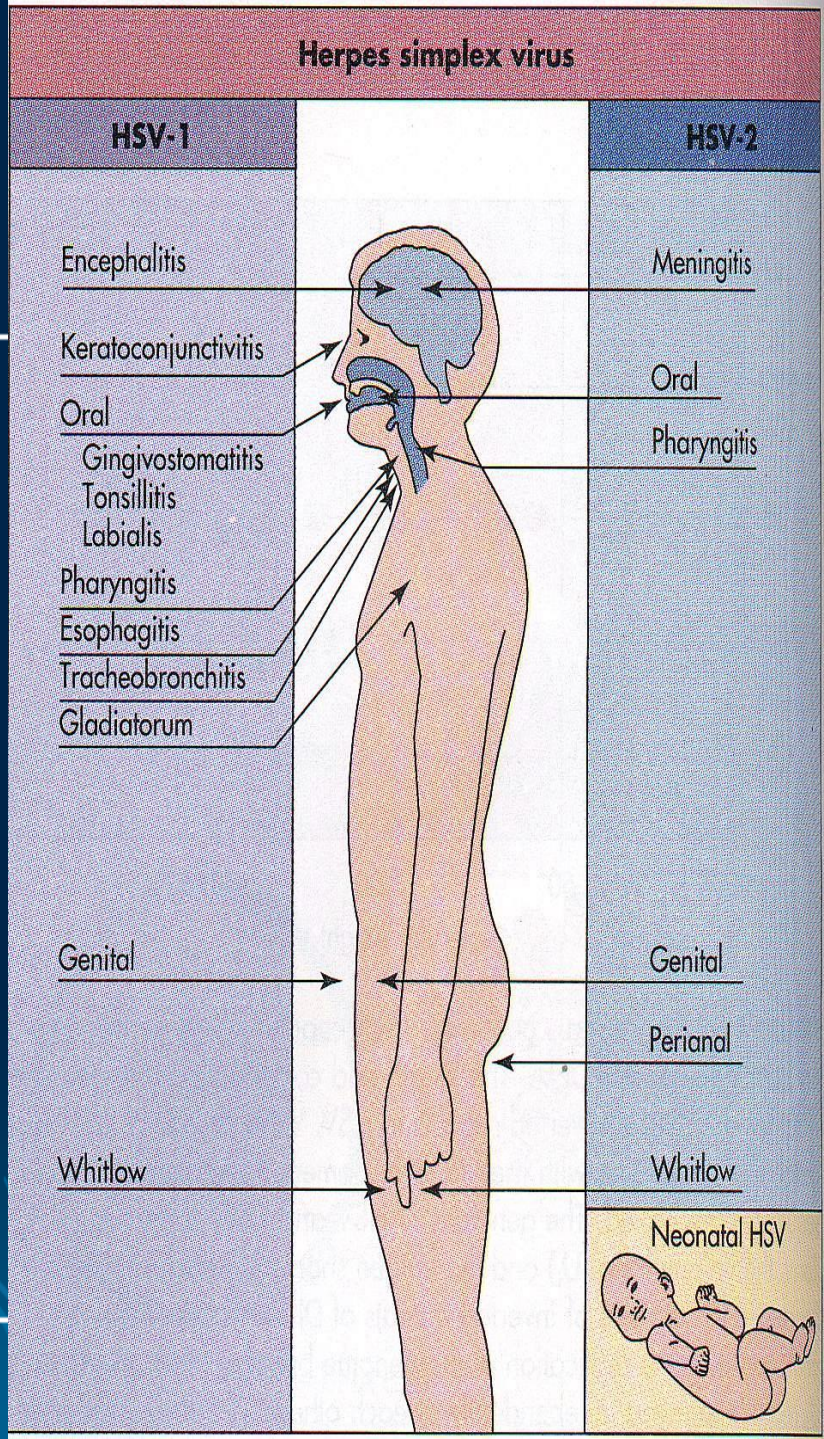
TOGAVIRIDAE
- **Eastern equine**
- **Western equine**
- **Venezuelan equine**

FLAVIVRIDAE
- **St. Louis**
- **West Nile**
- **Murray valley**
- **Powassan**
- **Japanese B**

BUNYAVIRIDAE
- **California**

Herpesviridae

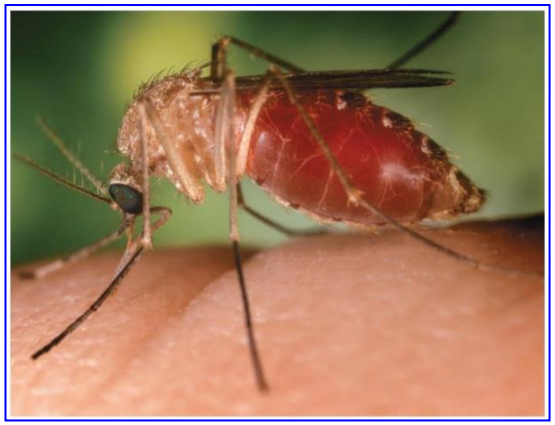
1-Herpes simplex virus type -1	HSV-1
2-Herpes simplex virus type -2	HSV-2
3-Varicella –Zoster virus	VZV
4-Epstein- Barr virus	EBV
5-Cytomegalovirus	CMV
6-Human herpes virus type-6	HHV-6
7-Human herpes virus type-7	HHV-7
8-Human herpes virus type-8	HHV-8



Herpes Simplex Encephalitis

- Herpes Simplex encephalitis is one of the most serious complications of herpes simplex disease. There are two forms:
- Neonatal – there is global involvement and the brain is almost liquefied. The mortality rate approaches 100%.
- Focal disease – the temporal lobe is most commonly affected. This form of the disease appears in children and adults. It is possible that many of these cases arise from reactivation of virus. The mortality rate is high (70%) without treatment.
- It is of utmost importance to make a diagnosis of HSE early. It is general practice that IV acyclovir is given in all cases of suspected HSE before laboratory results are available.

The term ARBO is an abbreviation of "**AR**thropod **BO**rne".



"Arbovirus" is the name given to Arthropod-borne viruses, that is, viruses that are transmitted to vertebrates, such as people and mammals, by blood-feeding insects called arthropods. Vertebrate infection occurs when the infected insect bites an animal or person and takes a blood meal.

Arboviruses

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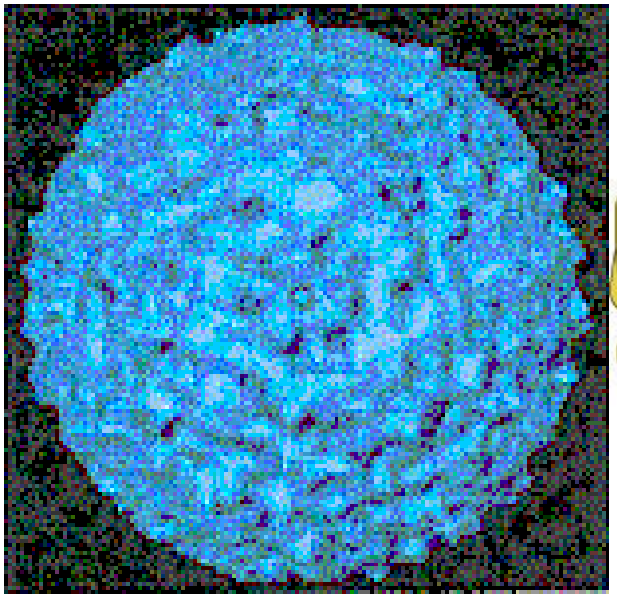
They can multiply in the tissues of the arthropod without evidence of disease or damage. The vector acquires a lifelong infection through the ingestion of blood from a viremic vertebrate.

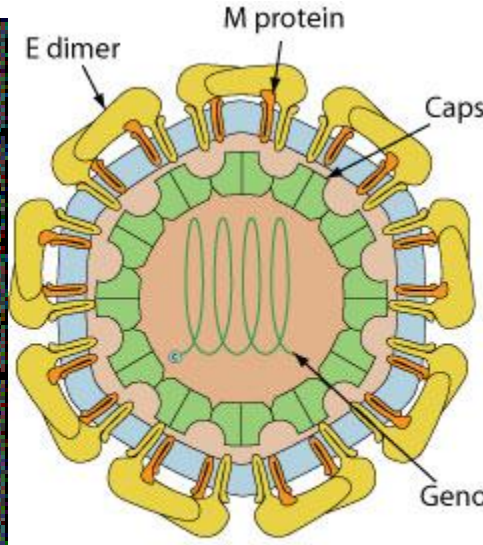
All arboviruses have an RNA genome, and most have a lipid-containing envelope and consequently are inactivated by ether or sodium deoxycholate.

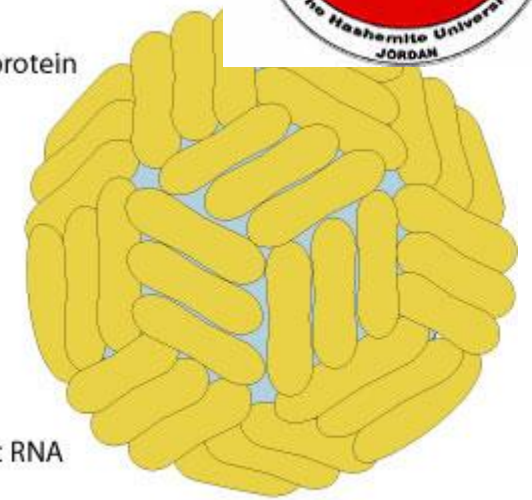
Diseases Caused

- Fever and rash - this is usually a non-specific illness resembling a number of other viral illnesses such as influenza, rubella, and enterovirus infections. The patients may go on to develop encephalitis or hemorrhagic fever.
- Encephalitis - e.g. EEE, WEE, St Louis encephalitis, Japanese encephalitis.
- Haemorrhagic fever - e.g. yellow fever, dengue, Crimean-Congo haemorrhagic fever.

Structure of Flaviviruses

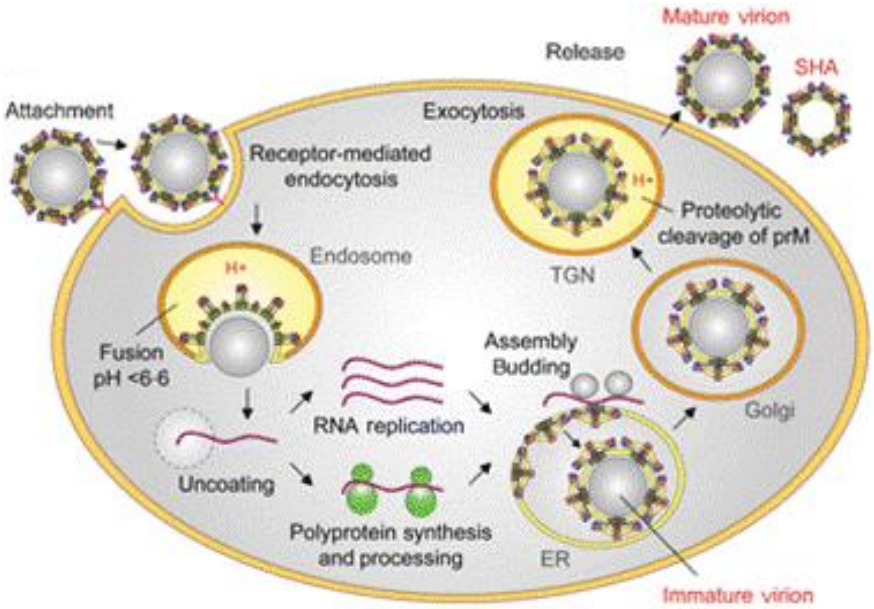






T=3-like organization of surface dimers

Positive sense, single stranded, enveloped RNA viruses with icosahedral capsid.



Principal medically important Flaviviruses

Virus	Antigenic Clinical Syndrome	Vector	Host	Distribution
Murray valley	Encephalitis	Mosquito	wild water birds	Australia
Powassan	Encephalitis	Tick	Squirrels snowshoe hare Rabbit	Canada
St. Louis encephalitis (SLE)	Encephalitis	Mosquito	Birds	Americas

Principal medically important Flaviviruses

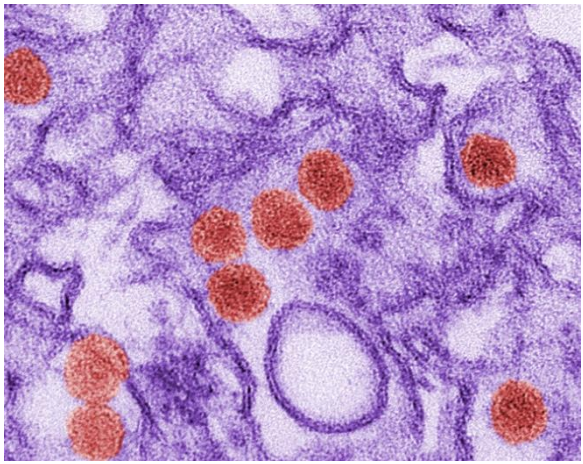
Virus	Antigenic Clinical Syndrome	Vector	Host	Distribution
Japanese encephalitis (JE)	Encephalitis	Mosquito	Pigs, birds	India, China, Japan, South-East Asia
West Nile	Febrile illness or encephalitis	Mosquito	Birds	Africa, Middle East, Europe
Tick-borne encephalitis (TBE)	Encephalitis	Tick	Rodent	Europa, Asia

Symptoms : West Nile virus

- Most people do not develop symptoms
- An estimated 20% become ill 3-15 days after being bitten
  - Mild illness: fever, headache, body aches, and sometimes skin rash and swollen glands
- An estimated 1 in 150 persons infected develop a more severe form of the disease
  - West Nile encephalitis: inflammation of the brain, high fever, stiff neck, stupor, disorientation, coma, tremors, convulsions, muscle weakness, and paralysis; few cases have been fatal

Zika Virus

- Genus *Flavivirus*
  - Closely related to West Nile Virus, dengue, Yellow Fever viruses
- Epidemiology
  - 1947: 1st isolated in Uganda
  - 2013: 1st large outbreak in French Polynesia (>28,000 cases)
  - 2015: Large outbreak in S. America
    - Over 30 nations reporting local transmission
    - >1.5 million cases estimated in Brazil alone

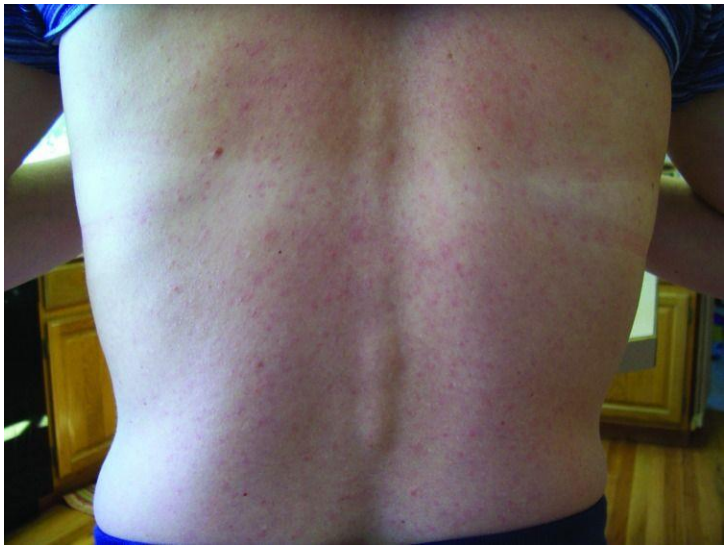


Transmission

- Mosquitos
  - *Aedes aegypti*
  - *Aedes albopictus*
  - Also transmit dengue and Chikungunya viruses
- Maternal-Fetal
  - Intrauterine and perinatal
  - Sexual transmission reported
  - Other possible routes
    - Bloodborne
    - Organ or tissue transplantation

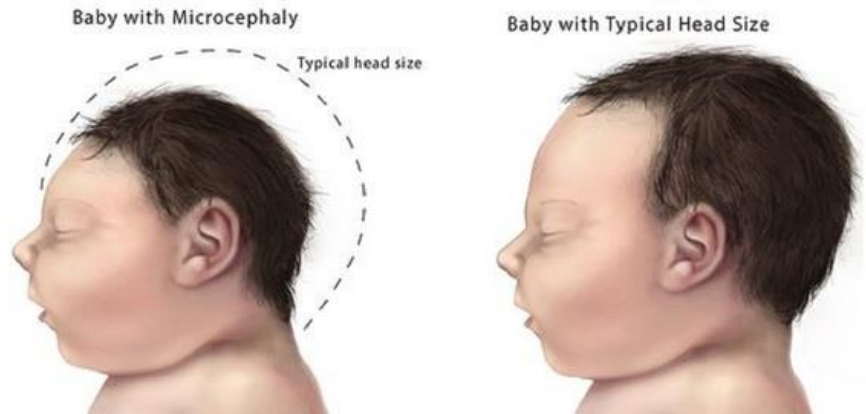
Zika Virus Clinical Disease

- ~80% of individuals are asymptomatic
- Clinical illness is usually mild
  - Fever
  - Conjunctivitis
  - Maculopapular rash
  - Myalgia and headache
- Symptom duration: 2-7 days
- Hospitalization is uncommon and fatalities are rare



Sequelae Associated with Zika Virus Infection

- Microcephaly
  - 20X increase in babies born with microcephaly in Brazil since start of Zika virus outbreak
  - Zika virus was confirmed in some of these infants
  - CDC recommendation to postpone travel if pregnant
- Guillain-Barré Syndrome (GBS)
  - Acute illness producing a lower, bilateral, symmetrical sensorimotor deficit.
  - Typically a history of infection prior to development of GBS
  - Incidence of GBS increased in all countries with Zika virus outbreak
  - Research ongoing to directly link GBS with Zika virus



Diagnosis, Treatment and Prevention

- Exposure history and laboratory testing
  - NAAT for detection of Zika virus RNA
    - Specimens: serum, amniotic fluid, CSF, tissue
    - Short duration of viremia (2-3 days)
  - Serology (IgM and IgG antibodies)
- Treatment:
  - Supportive care only
- Prevention:
  - Vaccine
  - Avoidance of mosquito bites

Diagnosis

- Serology - usually used to make a diagnosis of arbovirus infections.
- Culture - a number of cell lines may be used, including mosquito cell lines. However, it is rarely carried out since many of the pathogens are group 3 or 4 pathogens.
- Direct detection tests - e.g detection of antigen and nucleic acids are available but again there are safety issues.

Prevention

- Surveillance - of disease and vector populations
- Control of vector - pesticides, elimination of breeding grounds
- Personal protection - screening of houses, bed nets, insect repellants. When possible, wear protective clothing while outdoors.
- Vaccination - available for a number of arboviral infections e.g. Yellow fever, Japanese encephalitis, Russian tick-borne encephalitis

Treatment

- No specific therapy
- Arboviral encephalitis treated by hospitalization, intravenous fluids, respiratory support, prevention of secondary infections, and good nursing care
- Aspirin and ibuprofen should be avoided because they increase the risk of bleeding

Prions

Prions are rather ill-defined infectious agents believed to consist of a single type of protein molecule with no nucleic acid component.

Confusion arises from the fact that the prion protein & the gene which encodes it are also found in normal 'uninfected' cells.

These agents are associated with diseases such as Creutzfeldt-Jakob disease in humans, scrapie in sheep & bovine spongiform encephalopathy (BSE) in cattle.

Prion diseases: rare neurodegenerative disorders (one person per million)

1. Sporadic (85 %)

In the sixth or seventh decade, rapidly progressive (death in less than a year)

Creutzfeldt-Jakob disease (CJD)

2. Familial (inherited-15%)

Mutations in the PrP gene that favour the transition from the cellular form to the pathological form of PrP

Gerstmann-Straussler-Scheinker disease (GSS), fatal familial insomnia (FFI)

3. Transmissible (rare; a source of great concern)

Propagation of kuru disease in New Guinea natives (ritualistic cannibalism)

Recently, it has been discovered that BSE had been transmitted to humans in Europe after consumption of infected beef, producing a variant of the CJD called vCJD

Transmissible spongioform encephalop (TSE)=prion disease

A group of progressive conditions that affect the brain and nervous system of humans and animals and are transmitted by prions

The pathology: vacuolar degeneration, neuronal loss, astrocytosis and amyloid plaque formation

The clinical signs: loss of motor functions (lack of coordination, ataxia, involuntary jerking movements), personality changes, depression, insomnia, confusion, memory problems, dementia, progressive tonic paralysis, death

Definitive diagnostic test: biopsy of brain tissue (histopathological examination and immunostaining for $PrP^{Sc)}$

There is no cure

Prion transmission

1. Direct contact with infected tissues

CJD has been transmitted:

- To patients taking injections of growth hormone harvested from human pituitary glands
- From instruments used for brain surgery (prions can survive the autoclave sterilization process)
- In corneal grafts
- In electrode implants

2. Consumption of affected tissues

Kuru was transmitted through cannibalism in Papua New Guinea

Humans can contract the disease by consuming material from animals infected with the BSE (vCJD)

How can prions make their way through the gut and into the brain?

Proteins normally are digested down to amino acids in the gut

Hypothesis: They circumvent the normal process of intestinal absorption by passing into the the Gut-Associated Lymphoid Tissue (GALT)

Protein misfolding diseases

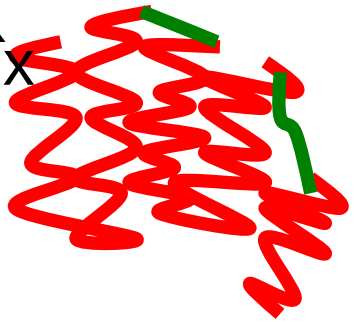
Arise from abnormal conformation of specific proteins

Principle: Proteins can adopt an aberrant conformation that cause disease; two mechanisms must be considered: loss of function of the native protein or gain of toxic activity of the aberrant conformation

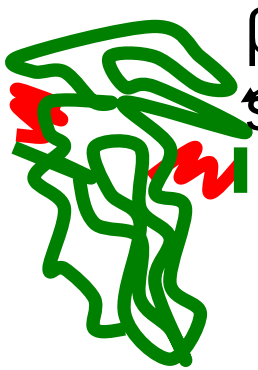
More than 20 human pathologies

Prion diseases arise from the harmful function of the abnormal proteins; misfolded forms of proteins (rich in β-sheet structures) have a strong propensity to aggregate into insoluble material and form fibrils

α-helix



Conformational change



β-sheet

Normal protein (folded structure)

Disease-associated protein (misfolded structure)

Aggregation

Gain of toxic activity

Loss of biological function

Genetics of prion disease

Familial forms of prion disease are caused by inherited mutations in the PRNP gene

Mutations in this gene cause cells to produce an abnormal form of the prion protein, known as PrP^{Sc}

Most cases of prion disease are sporadic, they occur in people without gene mutations

Familial forms of prion disease are inherited in an autosomal dominant pattern

PrP^{C}	PrP^{Sc}
The normal protein	The abnormal, disease-producing protein
is called PrP^{C} (for cellular)	is called PrP^{Sc} (for scrapie)
is a transmembrane glycoprotein (neurons, lymphocytes); its function is unknown	has the same amino acid sequence (primary structure)
has dominant secundary structure α-helix	has dominant secundary structure β-sheets
is easily soluble	is insoluble
is monomeric and easily digested by proteases	is multimeric and resistant to digestion by proteases
is encoded by a gene designated PRNP located on the chromosome 20	When PrP^{Sc} comes in contact with PrP^{C}, it converts the PrP^{C} into more of itself These molecules bind to each other forming aggregates

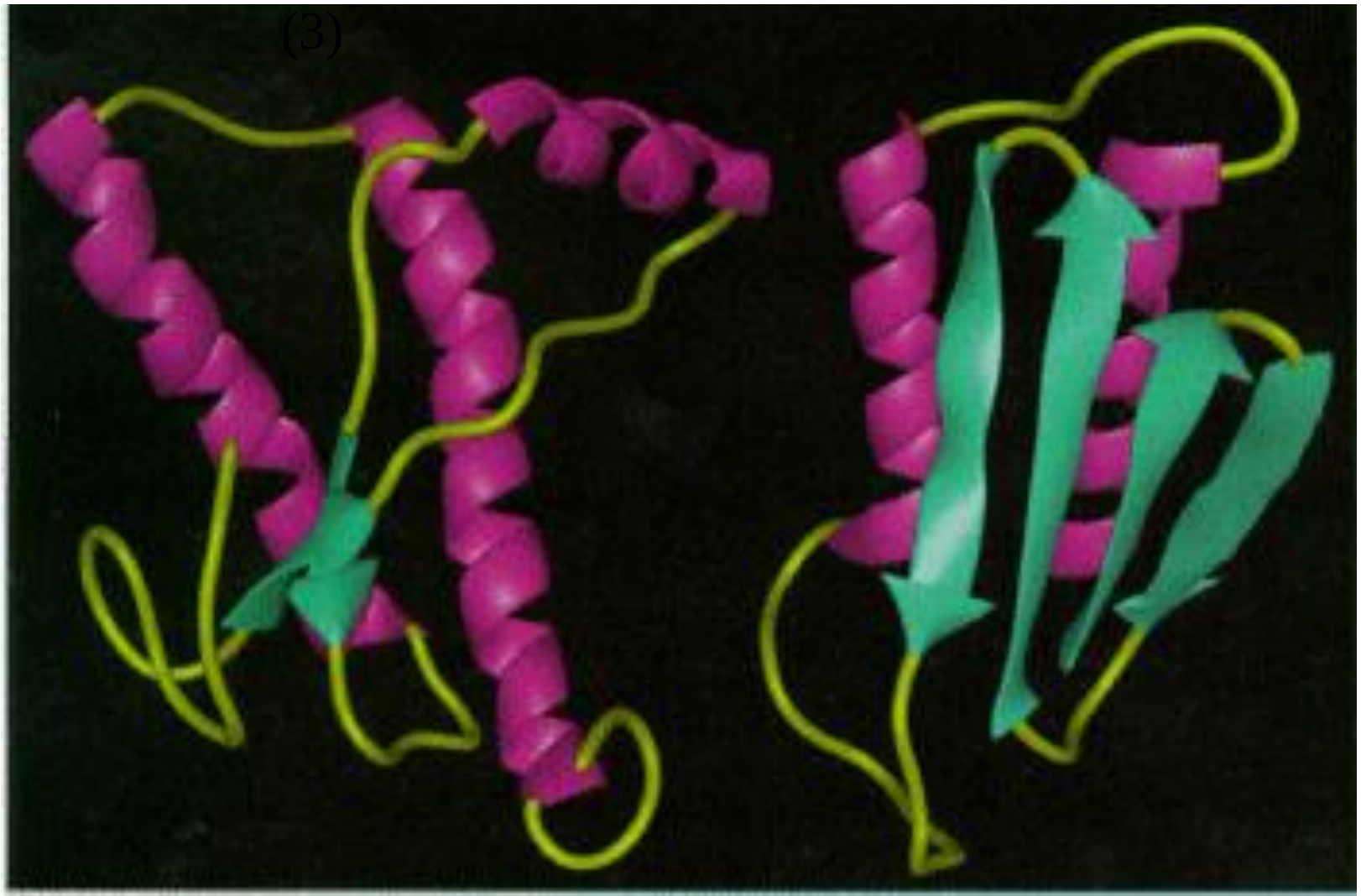
Molecular models of the structure of

PrP^{C}

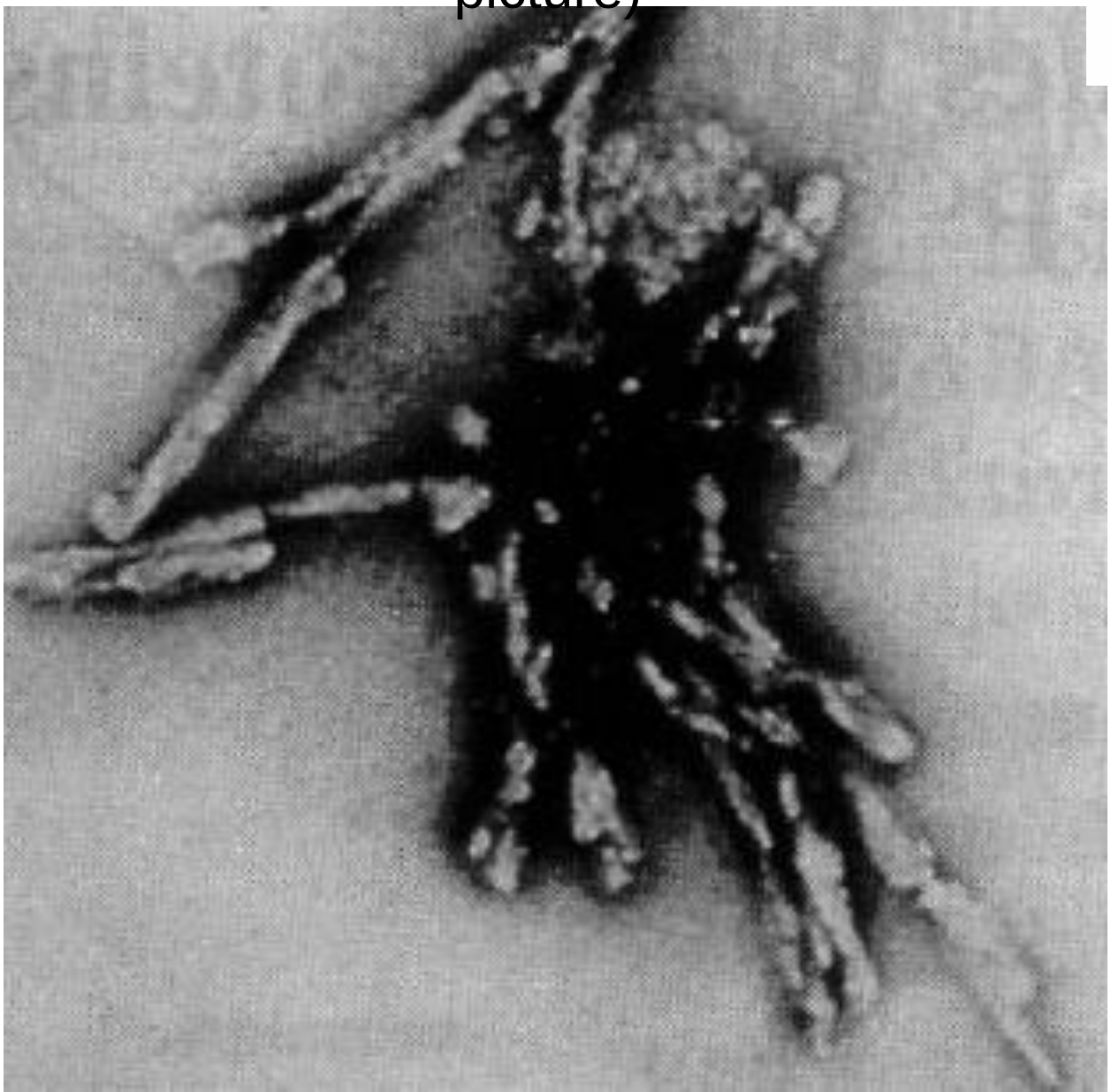
PrP^{Sc}

Predominantly α-helix

β-sheets (40%), α-helix (30%)



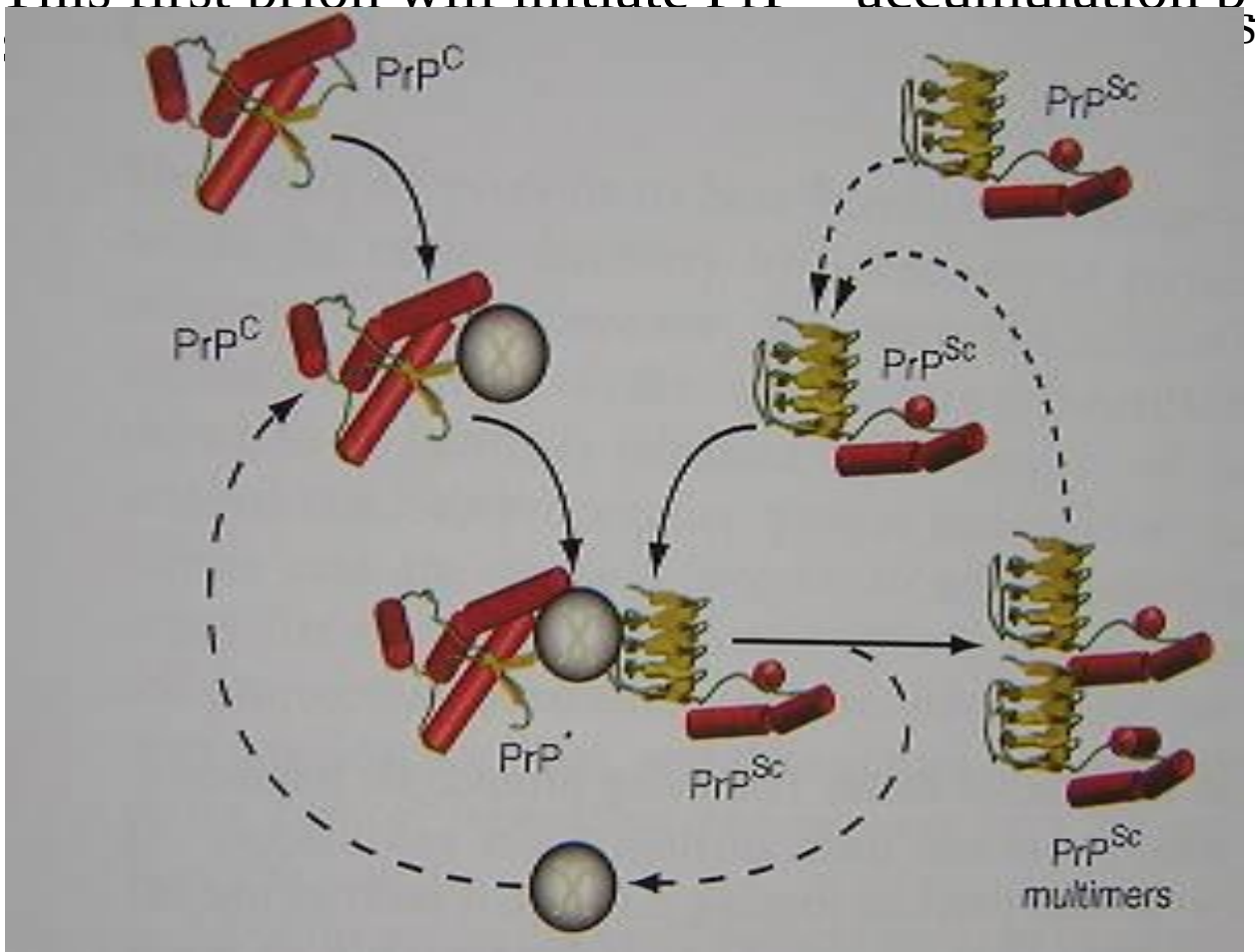
Prion aggregates (an electron microscope picture)



Replication cycle

The presence of an initial PrP^{Sc}: exogenous (infectious forms) or endogenous (inherited or sporadic forms)

This first prion will initiate PrP^{Sc} accumulation by



Kuru

(a native word meaning “trembling with cold and fever“)

Is a prion disease incident in natives in New Guinea

(first noted in the early 1900s)

In 1950-60 epidemic

Cannibalism: relatives ate their dead relative's brains as a sign of mourning

In the 1950's, the practice was banned, thereby preventing any further possible transmission; (incubation period of 4 to 20 years)

Symptoms: 3 stages; gradually deterioration of motor and mental functions

The first stage, exhibits unsteady gait, decreased muscle control, tremors, deterioration of speech and dysarthria (slurred speech).

second stage, incapable of walking without support, suffers ataxia (loss of muscle coordination), severe tremors and depression.

final stage, the patient suffers severe ataxia, is unable to speak, is incontinent, has dysphagia (starvation), is unresponsive to their surroundings

An infected person usually dies within 3 months to 2 years after the first symptoms, often because of pneumonia or pressure sores infection

Creutzfeldt-Jakob disease (CJD)

is the most common of the prion disease

usually affects people aged 55-65 (vCJD occurs in younger people)

The duration of CJD is less than 1 year

Symptoms: Dementia, hallucinations, motoric dysfunction, ataxia and seizure

Diagnosis: symptoms, EEG, MRI, CSF analysis

The definitive diagnostic test: biopsy of brain tissue

Treatment: fatal disease, searching for viable treatments

Forms:

1. Sporadic – most common
2. Familial – 5-10%
3. Transmitted: iatrogenic-iCJD - <1%

Blood donor restrictions: prions can be transmitted by blood transfusions; there is no test to determine if a blood donor is infected; restrictions for blood donors

Characteristic	Classic CJD	Variant CJD
Median age at death	68 years	28 years
Median duration of illness	4-5 months	13-14 months Max 2.5 years
Clinical signs and symptoms	Dementia; early neurologic signs	Prominent psychiatric/behavioral symptoms; delayed neurologic signs; hallucinations
Specific changes on MRI	Often present	Often present
Specific changes on EEG	Often present	Often absent
Immunohistochemical analysis of brain tissue	Variable accumulation of the PrP^{Sc}	Marked accumulation of the PrP^{Sc}
Presence of agent in lymphoid tissue	Not readily detected	Readily detected
Presence of amyloid plaques in brain tissue	Often present	Often present

Fatal familial insomnia

- **Cause:**
  - There is a mutation in PRNP (codes for prion protein) on chromosome 20. This mutation makes the protein insoluble.
  - When it converts, the protein causes plaque to form in the thalamus, which is the region responsible for regulation of sleep.
- **Inheritance:**
  - The dominant gene responsible for FFI has only been found in 28 families worldwide.
  - 5 of these are in the U.S.
  - If only one parent has the gene, the offspring have a 50% chance of getting the disease.

Fatal familial insomnia

- **Symptoms/Signs of the Disorder**
- Four stages:

1. Increasing insomnia, paranoia, phobias (4 months)
2. 2. Hallucinations (5 months)
3. 3. Complete inability to sleep, rapid weight loss (3 months)
4. 4. Dementia; person becomes unresponsive/mute (6 months)
5. Death occurs between 7 to 36 months from onset.

- **Treatment/Prevention:**
  - There is no cure for Fatal Familial Insomnia.
  - Gene therapy has been unsuccessful so far.
  - Sleeping pills don't help; they can actually speed disease progression.
  - Some scientists believe that a cure could be found in the next 10-15 years.
- **Prognosis**
  - Life expectancy ranges from 7 months to 6 years; with an average of 18 months

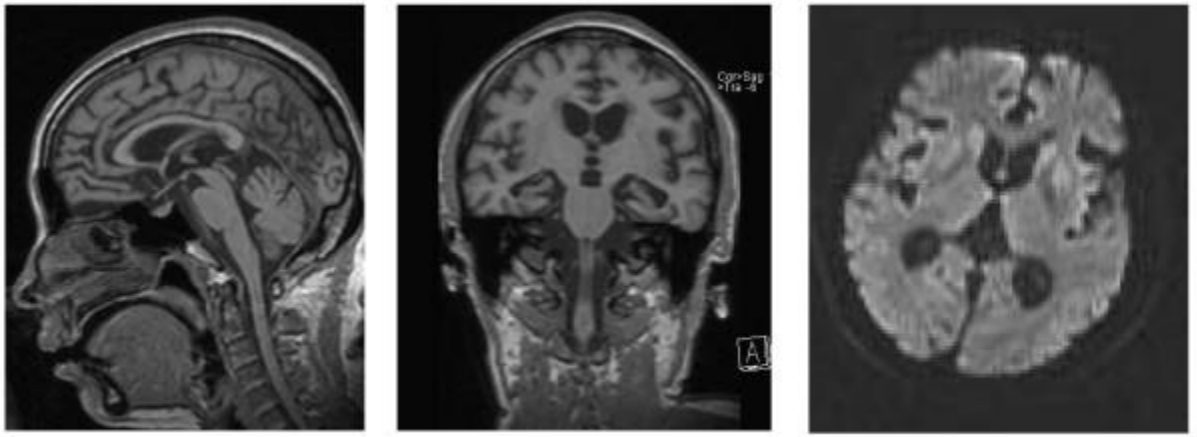
Gerstmann–Sträussler–Scheinker syndrome

- Cause:
  - There is a mutation in PRNP (codes for prion protein) on chromosome 20.
- Inheritance:
  - Autosomal-dominant gene
  - found in few families worldwide.

- Symptoms/Signs of the Disorder
  - slowly developing dysarthria (difficulty speaking)
  - cerebellar truncal ataxia (unsteadiness)
  - Progressive dementia
  - Loss of memory can be the first symptom of GSS.
  - Many patients also exhibit nystagmus (involuntary movement of the eyes), visual disturbances, and even blindness or deafness.

Gerstmann–Sträussler–Scheinker syndrome

- Diagnosis
  - Genetic testing to detect the mutated gene at certain codons.
- Treatment/Prevention:
  - There is no cure for GSS.
- Prognosis
  - Duration of illness can range from 3 months to 13 years with an average duration of 5 or 6 years



A B C

Diagnosis

- Gold standard: brain biopsy (histopathological examination and immunostaining for $PrP^{Sc)}$
- CSF: elevated protein 14-3-3 and S100
- CT and MRI: Normal, if abnormal not diagnostic
- EEG: abnormal pattern in 2/3 of Creutzfieldt-Jakob disease

Therapeutic strategies

1. Compounds can be designed to specifically disrupt the replication cycle of the PrP^{Sc}

Design of such compounds had proven successful in cell-based models but must now be extended to animal models and human clinical trials

2. Vaccine design: The abnormally folded proteins expose a side chain of amino acids which the properly folded protein does not expose. Antibodies specifically coded to this side chain amino acid sequence stimulate an immune response to the abnormal prions

3. Design of peptides that break the β-sheet structures

4. Gene therapy: modification of the prion gene

Genetic engineering research: cattle lacking a necessary gene for prion production - thus theoretically making them immune to BSE (December 2006)

Summary

The prions are proteins that carry information for self-reproduction (the central dogma of modern biology)

The prions are expressed in cells of healthy humans and animals; their abnormal conformations (PrP^{Sc}) are insoluble, resistent to digestion and aggregate

The PrP^{Sc} attacks the native prion PrP^{C}, changes its conformation into an abnormal form and causes an exponential production of insoluble proteins; they aggregate and form the fibrillar structure

Prion disease are rare fatal degenerative disorders; a portion of them can be transmitted; this mechanism is not clear (e.g. transmision of BSE to human)

One part of the prion protein can cause apoptosis, or programmed cell death

Prions induce no immune reactions within the human