Parkinson disease (PD)

- **second most frequent neurodegenerative disease (Alzheimer disease is the first)**

- **most frequent in terms of motor disorders**

- **<u>parkinsonism</u>, characterized by bradykinesia (or akinesia), postural rigidity/instability, tremors, altered autonomic functions and cognitive decline**

- **due to progressive degeneration of dopaminergic pigmented neurons (*substantia nigra*, basal nucleus: dopamin production, control of voluntary movements)**

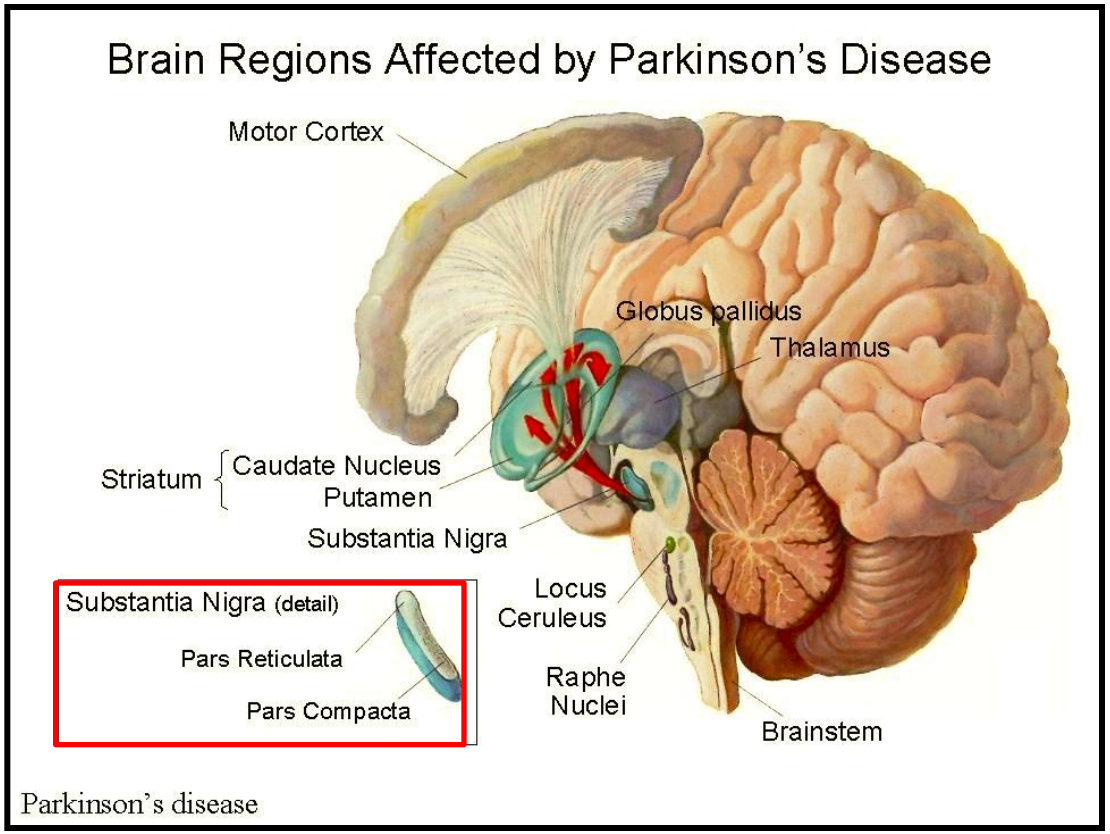
- **incidence increases with age:**
  65-69 yrs: 0,5-1,0%
  > 80 yrs: 1-3%

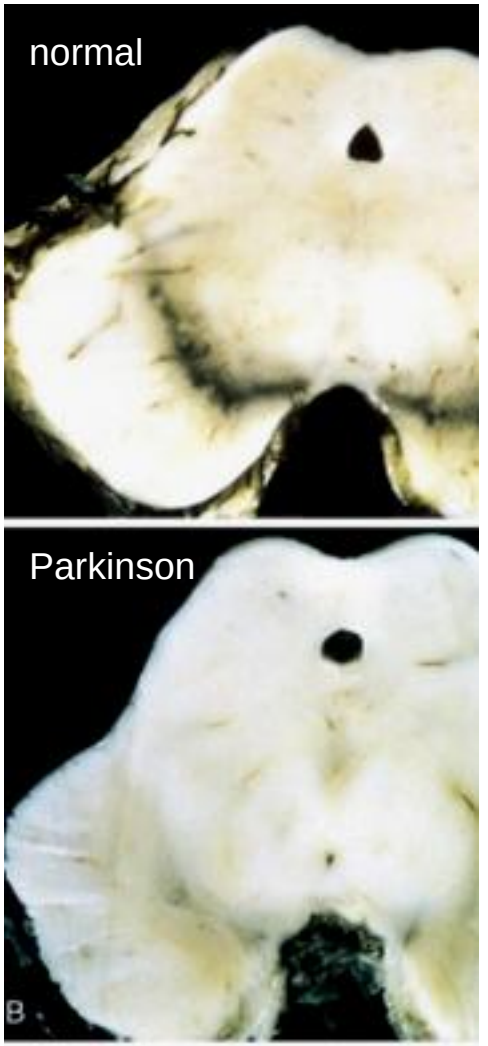
- **most frequent in males than in females**

Histopathology:

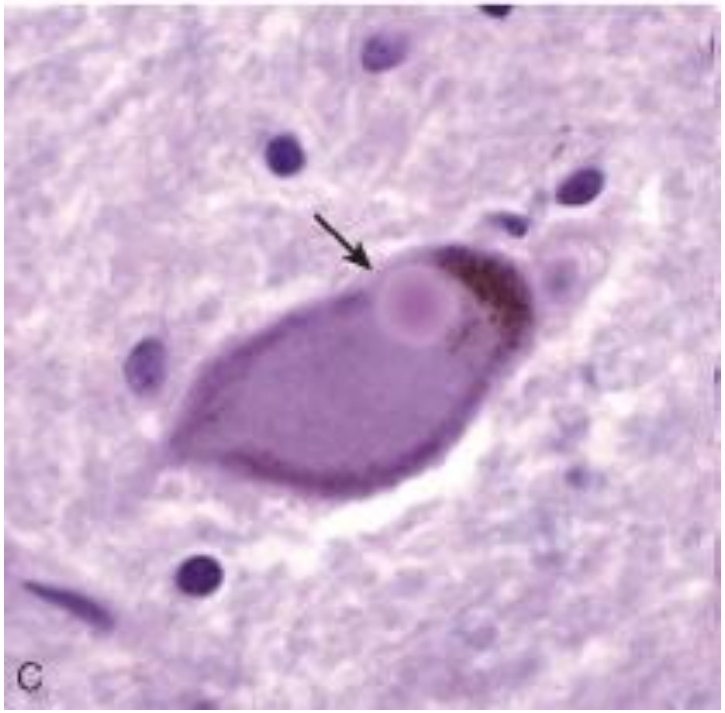
loss of dopaminergic neurons in *substantia nigra,*
Lewy bodies (cytoplasmic accumulation of ubiquitylated proteins in neurons).
Lewy neurites (filament inclusions)

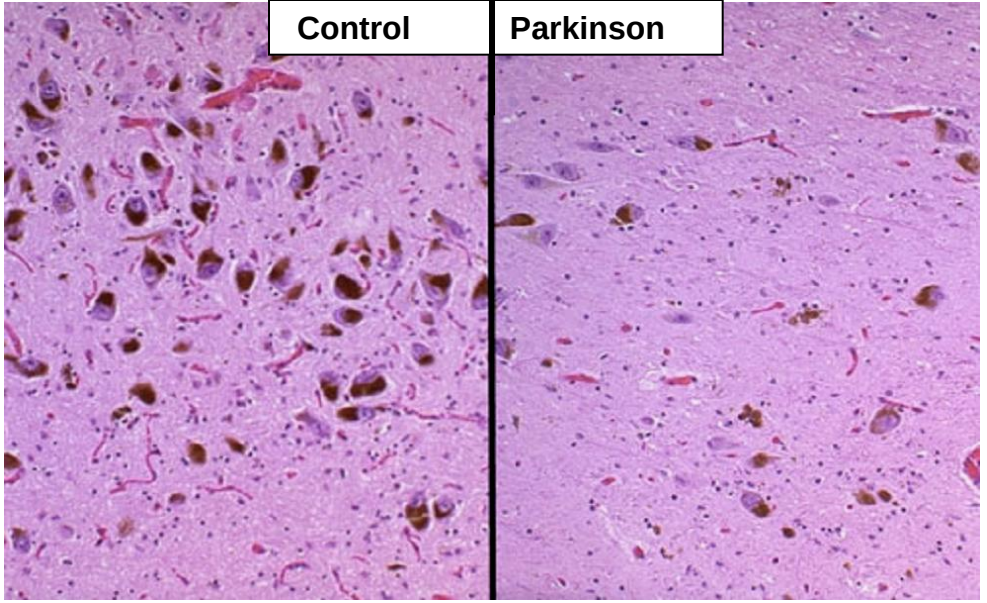
Initial lesions very confined



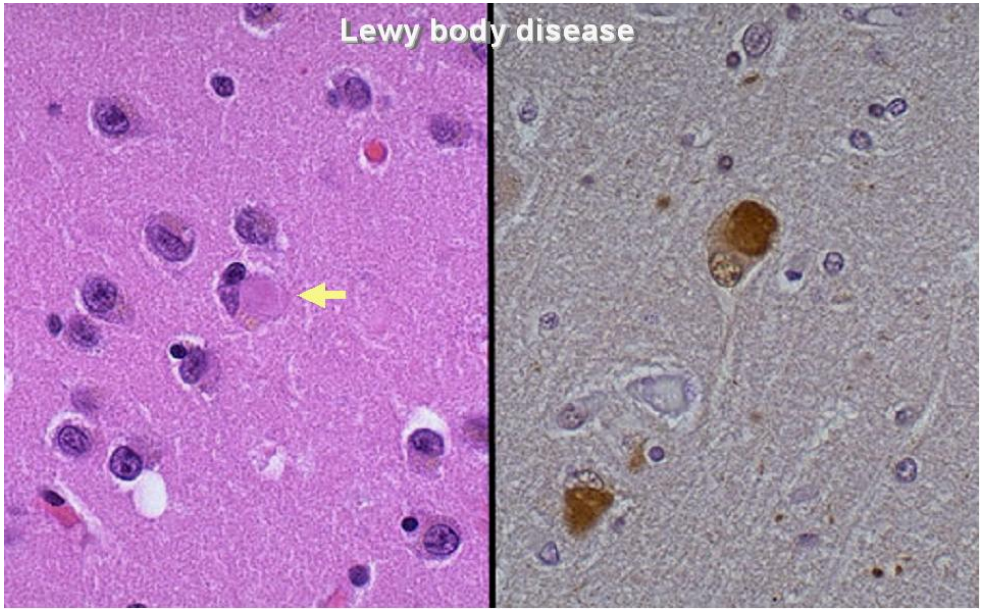


Lewy bodies: cytoplasmatic eosinophilic inclusions composed by misfolded **α-sinuclein** that is ubiquitylated but not degraded



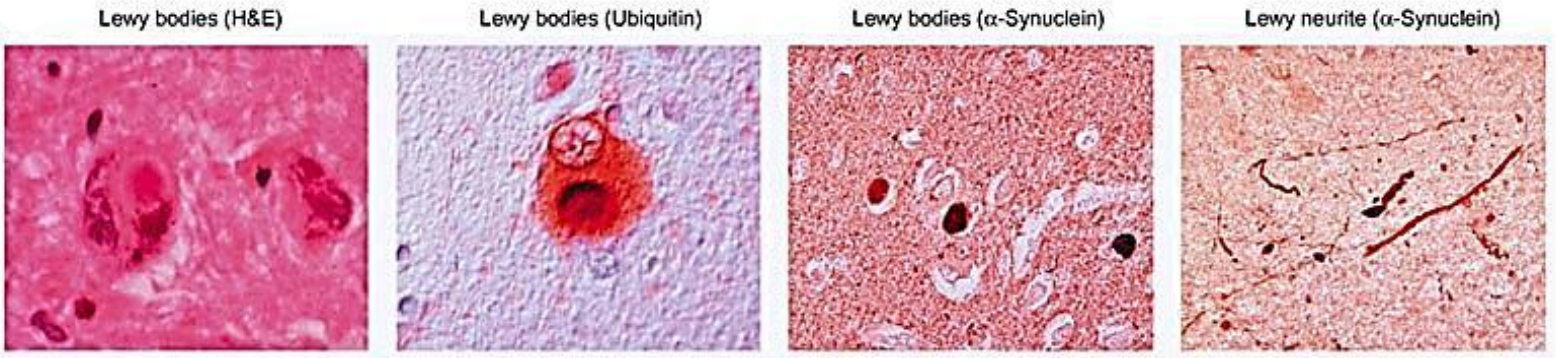


At the left, normal numbers of pigmented neurons in the subtantia nigra. At the right loss of neurons and loss of pigmentation with Parkinson's disease



At the left, an H&E stain demonstrates a rounded pink cytoplasmic Lewy body in a neuron of the cerebral cortex from a patient with diffuse Lewy body disease, which can be a cause for dementia. Lewy bodies can also be seen in substantia nigra with Parkinson's disease. An immunoperoxidase stain for ubiquitin, seen at the right, helps demonstrate the Lewy bodies more readily.

Parkinson disease



Histology related to PD.
Lewy bodies and neurites visualized by H&E staining or immunocytochemistry using antibodies specific for ubiquitin and α-synuclein in brain sections of sporadic cases of PD.

BOSSY-WETZEL 2004 *Nat Med* **10**, S2–S9

Dopamin

Binds specific receptors in different brain areas:
- nigrostriatal (movement control)
- memomimic-mesocortical (emotional control)
- infundibular, controls hormon release (es. GH and *Prolactin Inhibiting Factor)*

Produced by tyrosin hydroxilation to L-dopa then decarboxylated to dopamin

Dopamin → noradrenalin and adrenalin

After receptor interaction it is metabolized by
MAO (Mono-Amino-Oxydase) → 3,4-dihydroxy-phenylacetic acid
COMT(Cathecol-O-Methyl-Transferase) → 3-methoxy-tiramine

- **95% sporadic**

- **5% genetic (both AD and AR)**

AD forms
Point mutations or increased gene dose of α-sinuclein (gain of function)

AR forms, early onset
Loss of function mutations (*Parkin, PINK1, DJ-1*)

Lewy bodies and neurites

Lewy bodies (LBs) in neuron cell body
Lewy neurites (LNs) in neuronal processes

Uncertain meaning (neuronal death contribution?)

Number of LBs containing neurons is constant (3-4% in every disease stage)

Both LBs and LNs contain aggregated misfolded proteins, mainly **α-sinuclein**

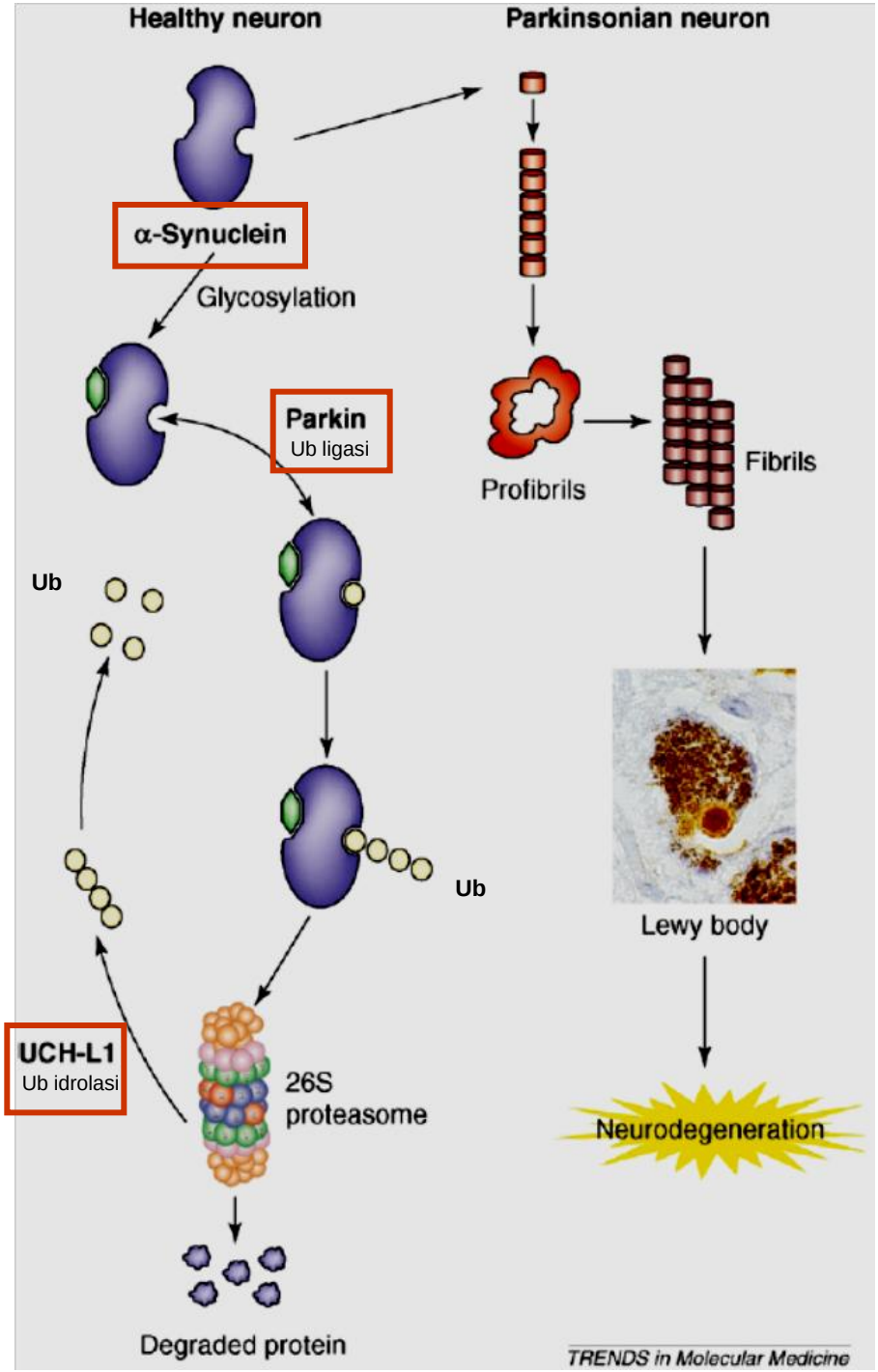
α-sinuclein

usually abundant in nervous processes and in synapses, increases in *substantia nigra* neurons in aging and PD

rapid turnover: glycosylation, ubiquitylation (parkin), proteasomal and/or autophagic degradation (autophagy degrades oligomers)

Mutations or other causes (es. oxidative stress) lead to accumulation

ACCUMULATION DUE TO DEGRADATIVE DEFICIT


Healthy neuron
Parkinsonian neuron
α-Synuclein
Glycosylation
Parkin
Ub ligasi
Profibrils
Fibrils
Ub
Ub
Lewy body
UCH-L1
Ub idrolasi
26S
proteasome
Neurodegeneration
Degraded protein
TRENDS in Molecular Medicine

PATHOGENESIS

No unifying mechanism, but many hypothesis

1. Misfolded stress response (**α-sinuclein**)
2. Proteasome degradation deficit due to loss of **parkin**
3. altered mitochondrial function (**DJ-1 and PINK1 loss**)

Syndrome severity associated to dopamina reduction. L-DOPA replacement therapy just symptomatic

Risk factors (epidemiology): pesticide exposure

PD-associated genes

5% of PD cases, 10 loci identified, 5 mutations are the most frequent

•locus PARK1 cr 4q21 ***SNCA*** → **α-sinucleina**. Rare mutations, PD associated with increased gene dosage due to promotor polymorphisms (altered expression)

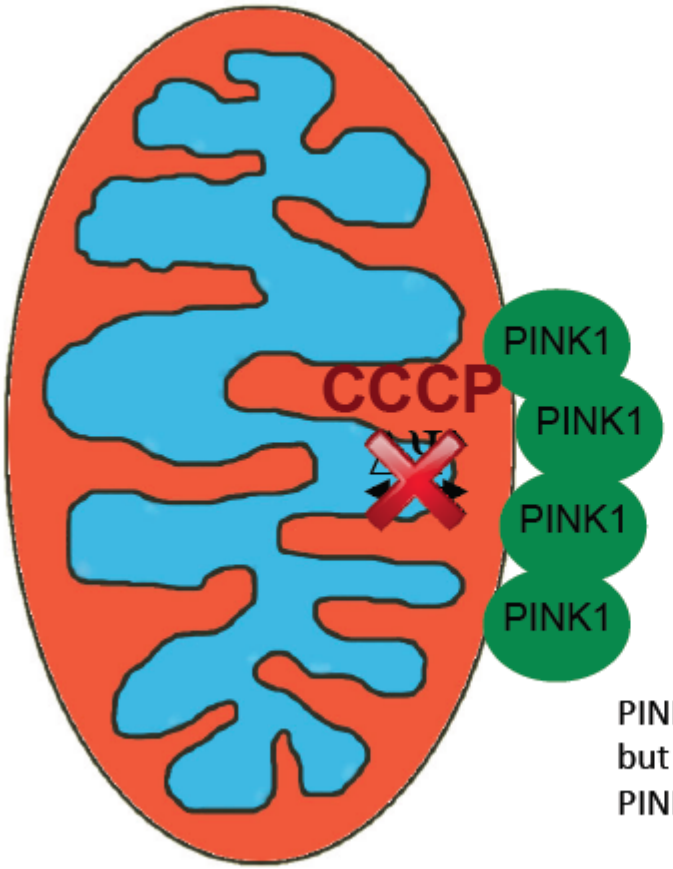
•***LRRK2*** (leucine-rich repeat kinase 2), in some AD forms

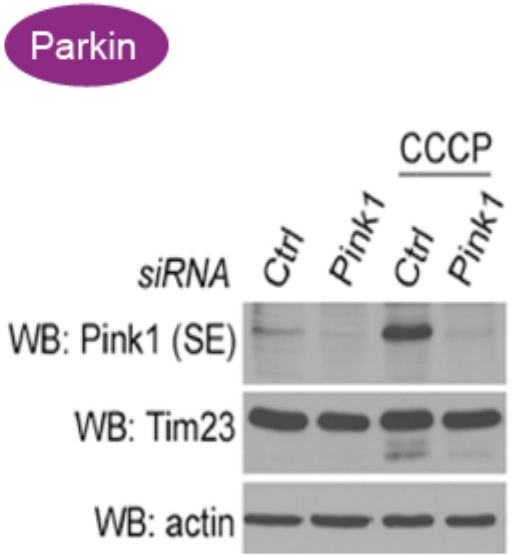
•locus **PARK2** *parkin* (E3 Ub-ligasi; no LBs), early onset AR

•***DJ-1*** (regulates redox balance) or **PINK1** (kinase involved in mitochondrial function regulation), both associated with AR forms

	Locus	MOI	Gene (protein)	Protein function	Age at onset
*	*PARK1* (*PARK4*)	AD	*SNCA* (α-synuclein)	Unknown synaptic function	Mid 30–mid 60 Mid 20–30 30–60
*	*PARK2*	AR	Parkin	E3 ubiquitin ligase	Juvenile to 40
	PARK5	AD	*UCHL1*	Ubiquitin hydrolase and ligase	30–50
*	*PARK6*	AR	*PINK1*	Mitochondrial Ser–Thr kinase	30–50
*	*PARK7*	AR	*DJ-1*	Oxidative stress response?	20–40
	PARK8	AD	*LRRK2* (dardarin)	Unknown protein kinase	40–60

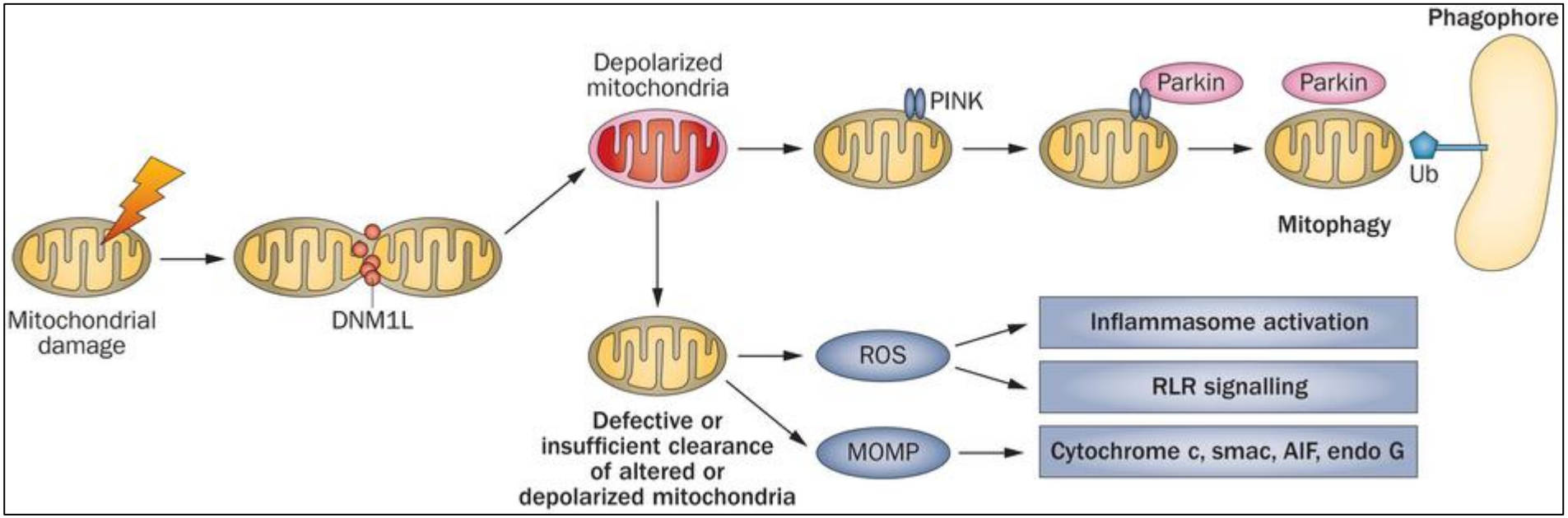
2- Depolarized mitochondria⇒ PINK1 stabilized



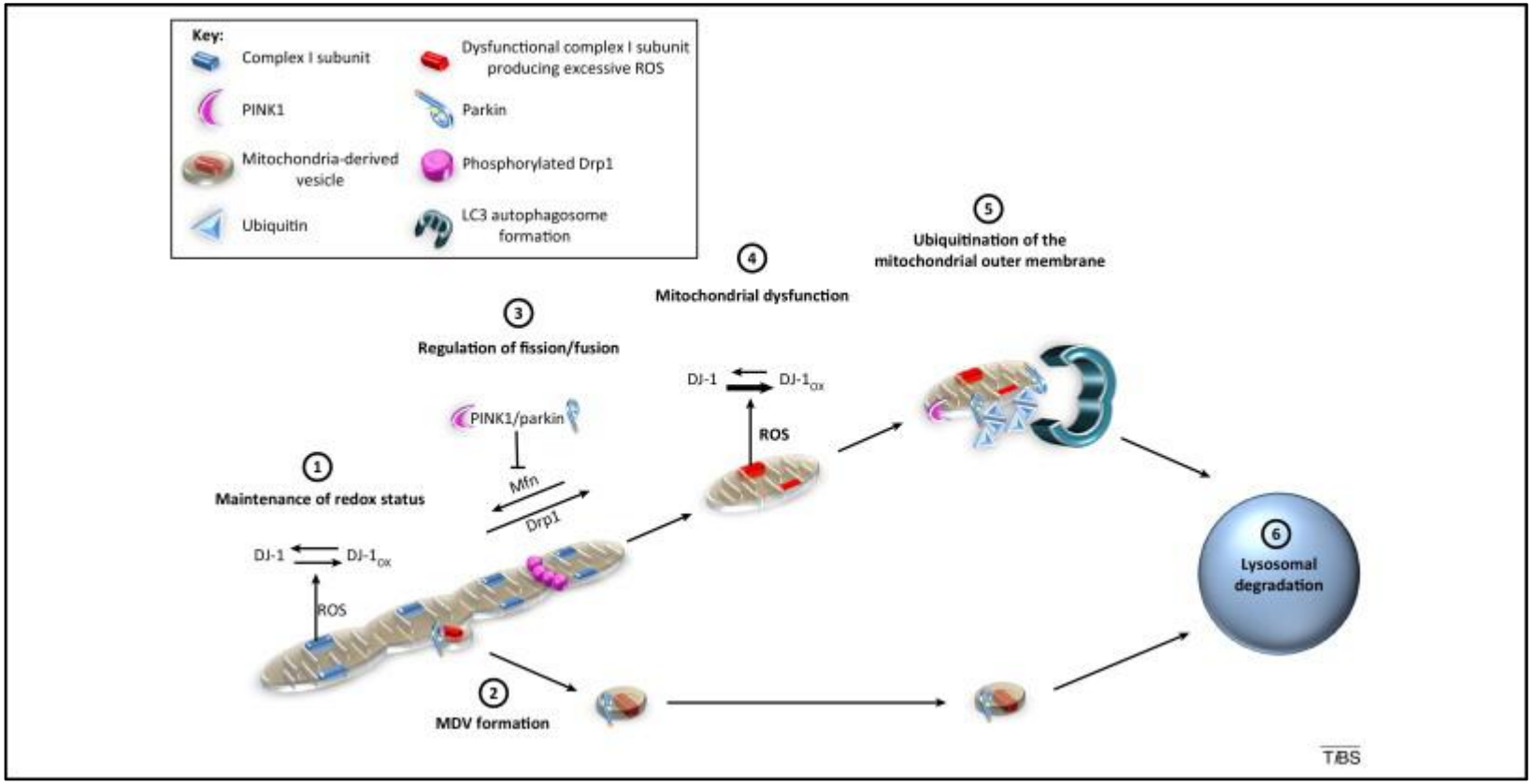


PINK1 is degraded rapidly in cells at steady-state but accumulates with depolarization (CCCP). PINK1 siRNA confirms specificity of antibody

http://edwardfon.lab.mcgill.ca/fr/index.php?page=1-2



Damaged mitochondria undergo DNM1L-mediated fission. Reduced membrane potential of depolarized mitochondria leads to PINK1 accumulation and subsequent recruitment of the E3 ubiquitin ligase Parkin to the mitochondria. Parkin-mediated ubiquitination of proteins in the mitochondrial membrane targets the damaged mitochondrion for removal by an autophagosome. Depolarized mitochondria that are not cleared by autophagy undergo MOMP, which leads to the release of catabolic hydrolases (AIF and endonuclease G) and caspase activators (cytochrome c and Smac) into the cytoplasm and induction of apoptosis. Mitophagy, therefore, delays intrinsic apoptosis by limiting the release of these pro-apoptotic factors. Excessive ROS production by depolarized mitochondria also enhances RLR signalling and promotes inflammasome activation. Abbreviations: AIF, apoptosis-inducing factor; DNM1L, dynamin-1-like protein (also known as dynamin-related protein 1); Endo G, endonuclease G, mitochondrial; IFN, interferon; MOMP, mitochondrial outer membrane permeabilization; Parkin, E3 ubiquitin-protein ligase parkin; PINK1, serine/threonine-protein kinase PINK1, mitochondrial; RLR, RIG-like receptors; ROS, reactive oxygen species; Smac, second mitochondria-derived activator of caspases; Ub, ubiquitin (Fougeray and Pallet, 2015).



Overview of the roles of Parkinson's disease (PD)-associated mitochondrial proteins in mitochondrial homeostasis and mitophagy. **(1)** DJ-1 acts as a redox sensor and antioxidant in mitochondria during normal homeostasis. **(2)** Upon oxidative stress, selective sequestration of mitochondrial components into phosphatase and tensin homolog deleted on chromosome 10 (PTEN)-induced putative kinase 1 (PINK1)/parkin-dependent mitochondria-derived vesicles (MDVs) occurs. These MDVs, which can fuse with lysosomes, likely act as an important mitochondrial quality-control mechanism. **(3)** Mitochondrial dynamics are regulated by proteins including mitofusins (Mfns) that promote fusion and dynamin related protein-1 (Drp1), which promotes fission. Mitochondrial fission as a result of phosphorylation (activation) of Drp1 leads to increased fragmented mitochondria, which generate more reactive oxygen species (ROS) and less ATP. Increased ROS production causes post-translational modification of proteins including oxidation of DJ-1, promoting mitochondrial fission and degradation. DJ-1 mutations lead to excessive fission/degradation. **(4)** Increased ROS production by damaged mitochondria (particularly complex I) results in increased DJ-1 oxidation. **(5)** Loss of mitochondrial membrane potential results in PINK1-dependent recruitment and activation of parkin. Parkin acts as an E3 ligase ubiquitinylating mitochondrial proteins, particularly outer membrane proteins, resulting in sequestration in autophagic vacuole; and **(6)** degradation of cargo by lysosomal proteases (Ryan et al., 2015).

PRIONIC DISEASES

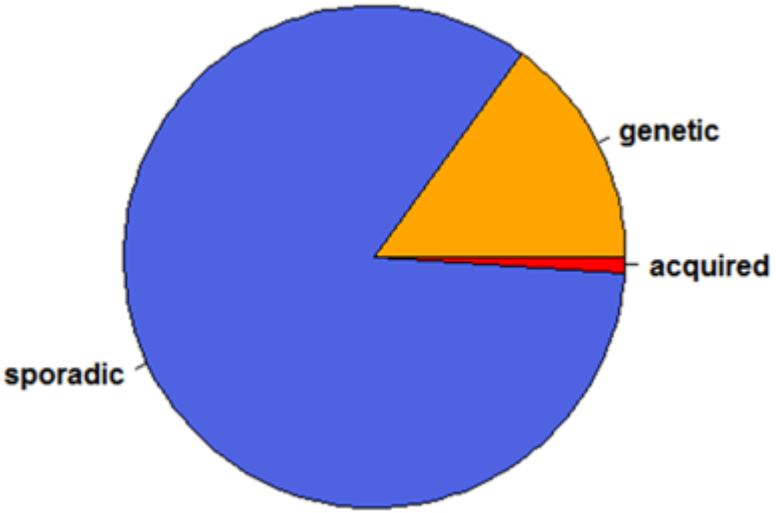
- **fatal outcome in both human beings and animals. Etiology can be sporadic, genetic or acquired**

- **animal-human and human-human transmission very rare**

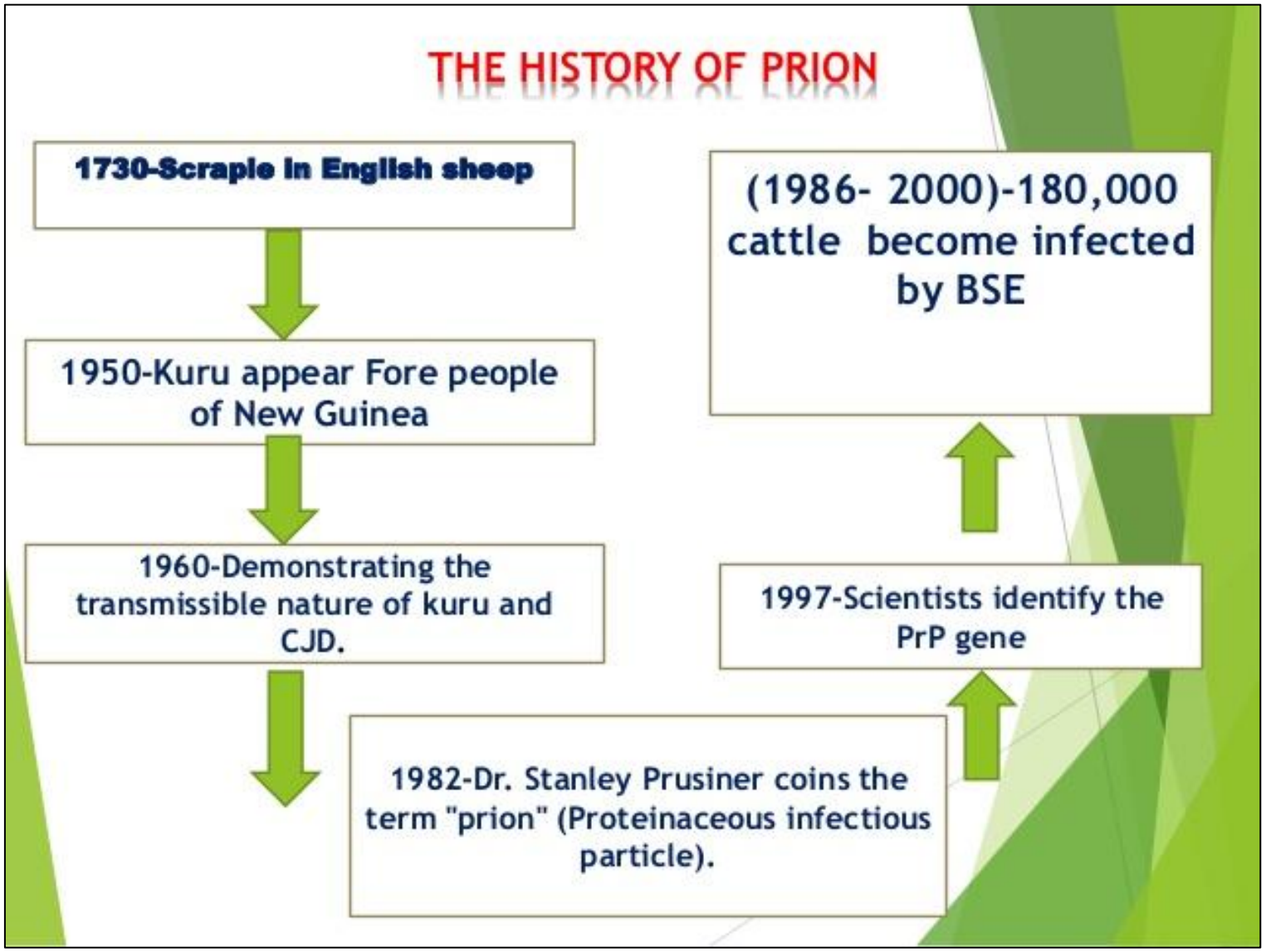
- **infective agent: PrP^{C} protein → conformational change to $PrP^{SC/TSE}$ associated to disease**

- **neuronal damage as hallmark of prionic diseases, unclear pathogenetic mechanisms**

- **intracellular PrP accumulation can alter neuronal function, mechanism of cytotoxicity of extracellular accumulation is unclear**



PrP^{C} normal form (C=cellular); $PrP^{SC/TSE}$ altered form (SC=scrapie/TSE=transmittable spongiform encephalopathy)

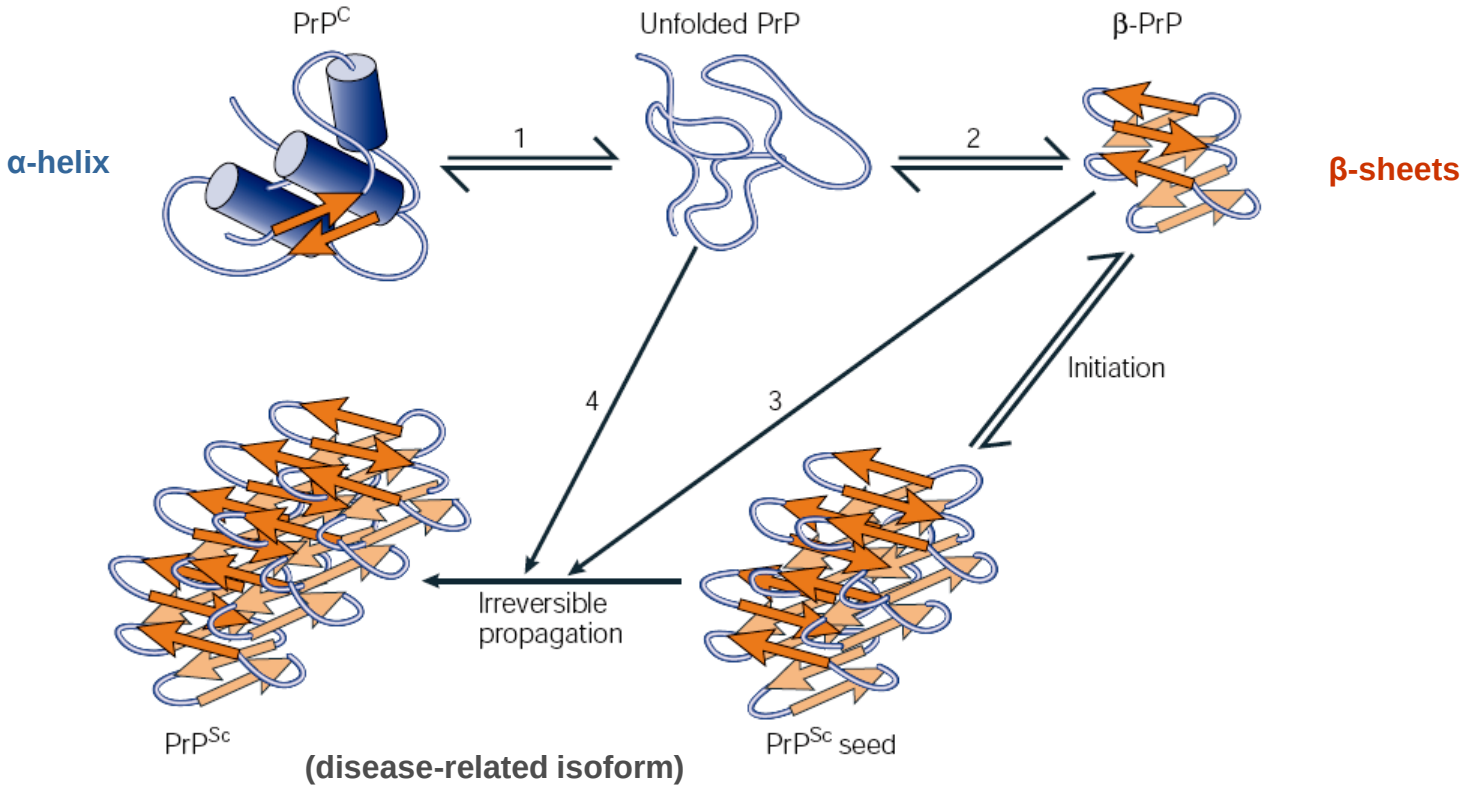

THE HISTORY OF PRION
1730-Scrapie in English sheep
1950-Kuru appear Fore people of New Guinea
1960-Demonstrating the transmissible nature of kuru and CJD.
1982-Dr. Stanley Prusiner coins the term "prion" (Proteinaceous infectious particle).
1997-Scientists identify the PrP gene
(1986- 2000)-180,000 cattle become infected by BSE

Classification of the human prion diseases

Aetiology	Phenotype	Frequency
Sporadic		
Apparently random distribution with an annual incidence of 1–2 per million worldwide	Sporadic CJD: multiple distinct prion strain types associated with distinct clinicopathological phenotypes; rarely associated with sporadic fatal insomnia	85%
Inherited		
Autosomal dominant with high penetrance; all are associated with *PRNP*-coding mutations	Highly variable: more than 30 mutations identified; includes GSS disease, familial CJD and fatal familial insomnia phenotypes	~10–15%
Acquired		
Iatrogenic exposure to human prions from medical contact with human cadaveric-derived pituitary hormones, tissue grafts or contaminated neurosurgical instruments	Iatrogenic CJD: typical CJD phenotype following direct CNS exposure; ataxic onset following peripheral infection	<5% (most patients from USA, UK, France and Japan)
Dietary exposure to human prions through endocannibalism	Kuru	Only in a small area of Papua New Guinea; epidemic in the 1950s, with a gradual decline after the cessation of cannibalism
Environmental exposure (presumed to be dietary) to the BSE prion strain	Variant CJD	Mainly in the UK (total so far ~150), 6 in France, individual patients in several other countries

BSE, bovine spongiform encephalopathy; CJD, Creutzfeldt–Jakob disease; GSS, Gerstmann–Sträussler–Scheinker; *PRNP*, the gene that encodes the prion protein.

A model of prion propagation



Such a model can accommodate the different aetiologies of human prion diseases:

- **acquired prion disease: prion propagation might be initiated by the introduction of a seed;**
- **sporadic prion disease: by spontaneous seed production as a rare stochastical event that involves wild-type PrP;**
- **inherited prion disease: PrP contains a pathogenic mutation.**

Following such initiating events, the process of propagation is driven thermodynamically by intermolecular association.0

Mallucci & Collinge 2005 *Nature Rev Neurosci* 6:23

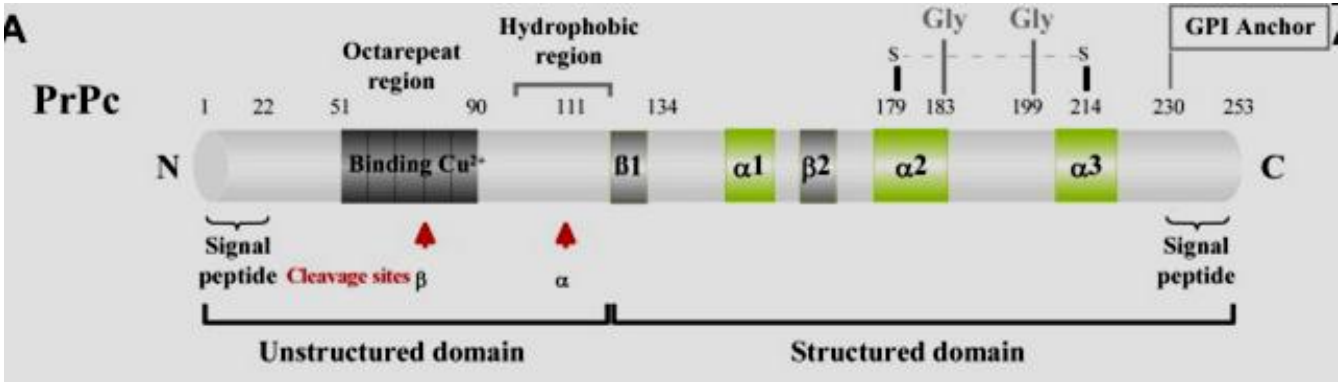
The cellular prion protein PrP^C

1. PrP^C **highly conserved and expressed in humans and various animals, largely as a glycoprotein.**

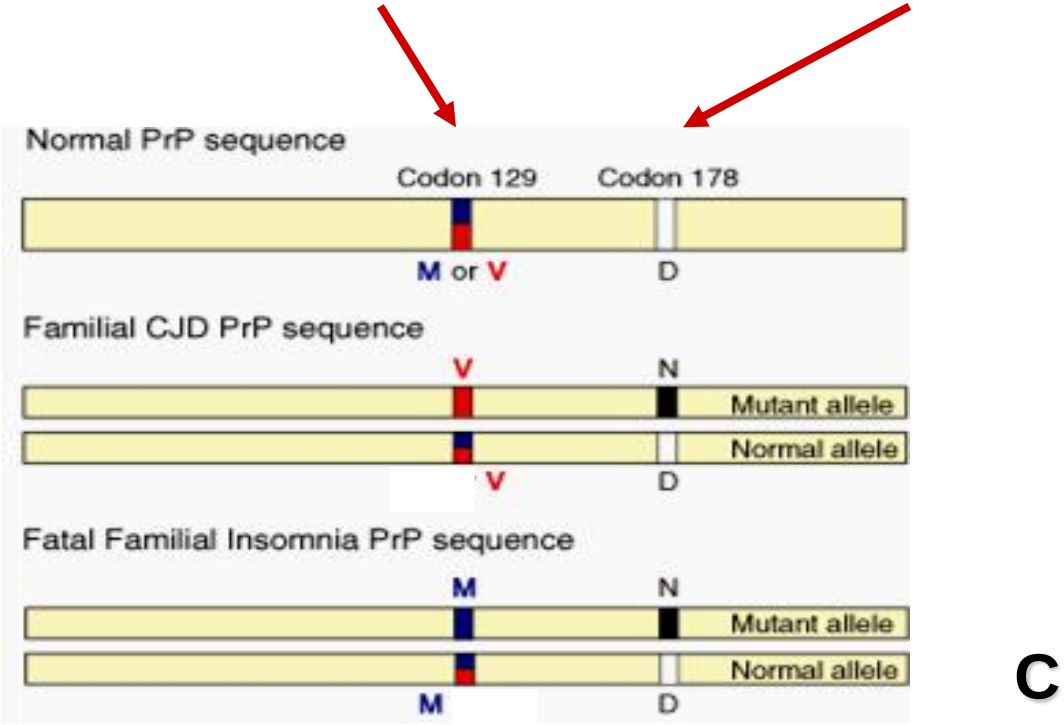
2. **Broad expression pattern: skeletal muscle, kidney, heart, secondary lymphoid organs and CNS.**

   In CNS: high PrP^C levels in synaptic membranes of neurons and on astrocytes.
   In the periphery: PrP^C on lymphocytes, follicular dendritic cells and erythrocytes.
   PrP^C also as a solute in body fluids such as blood plasma, and in milk.

3. **Human PrP, 253 amino acids (30 kDa), encoded by *PRNP*, single exon gene on p20.**
   23 aa on N terminus of PrP^C = signal peptide that targets the protein to the plasma membrane.
   In the plasma membrane, the C terminus is attached to a glycosyl phosphatidylinositol (GPI) anchor.
   It is plausible that PrP^C interacts with protein complexes on plasma membranes.



Codon 129 polymorphism & codon 178 mutations



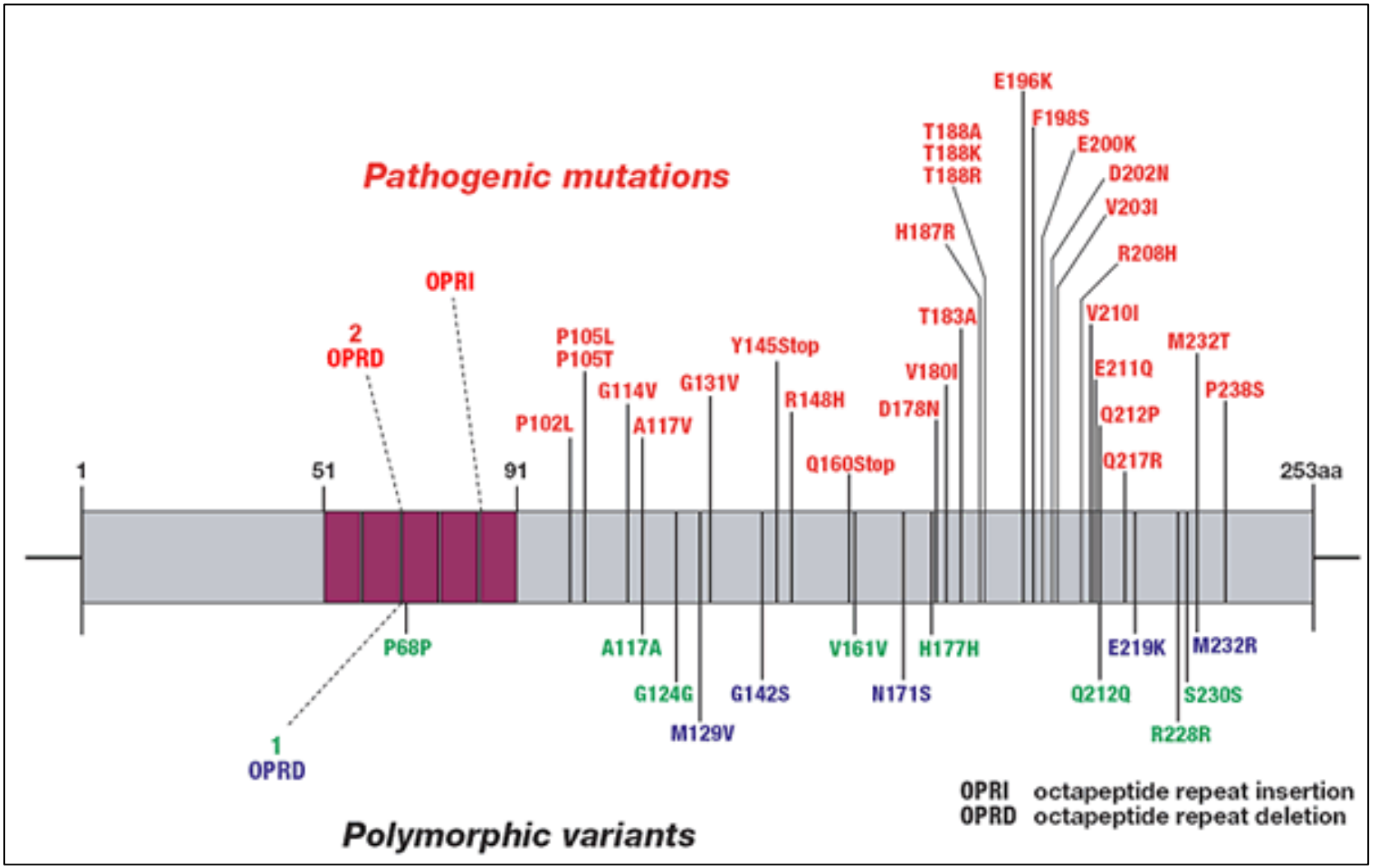
Sites of mutation (codon 178) and disease-associated polymorphism (codon 129)

In normal individuals: codon 178 encodes Asp (D), and codon 129 encodes either Met (M) or Val (V).

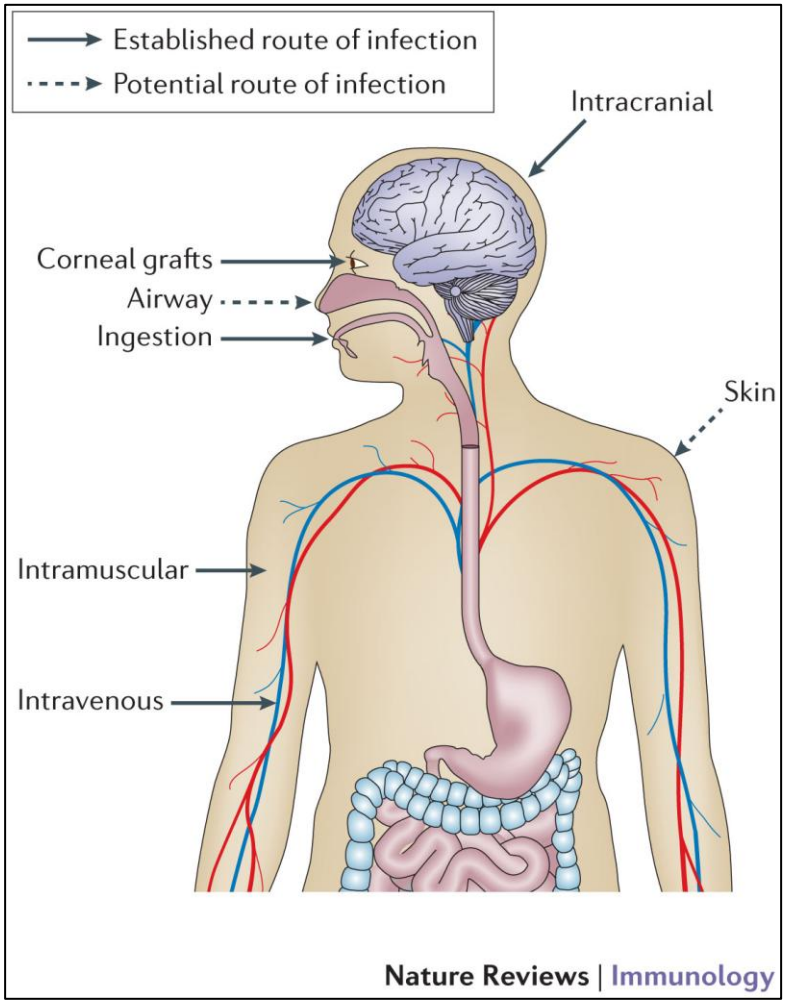
In some familial forms of disease, the mutation changes **codon 178 to Asn (D178N).**

- When the allele containing the D178N mutation also has a Val at codon 129, the patient develops **Creutzfeldt-Jakob disease (CJD).**
- In contrast, when the D178N allele has Met at codon 129, the clinical disorder is **fatal familial insomnia (FFI).**

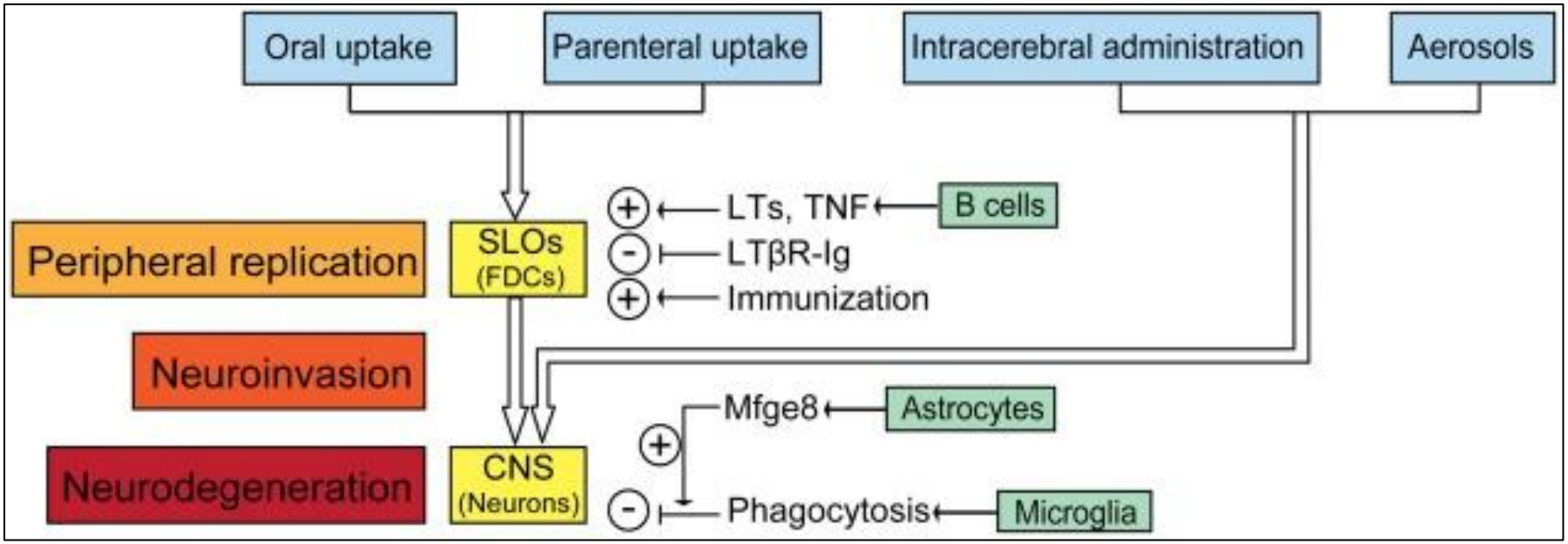
D = Asp; N = Asn



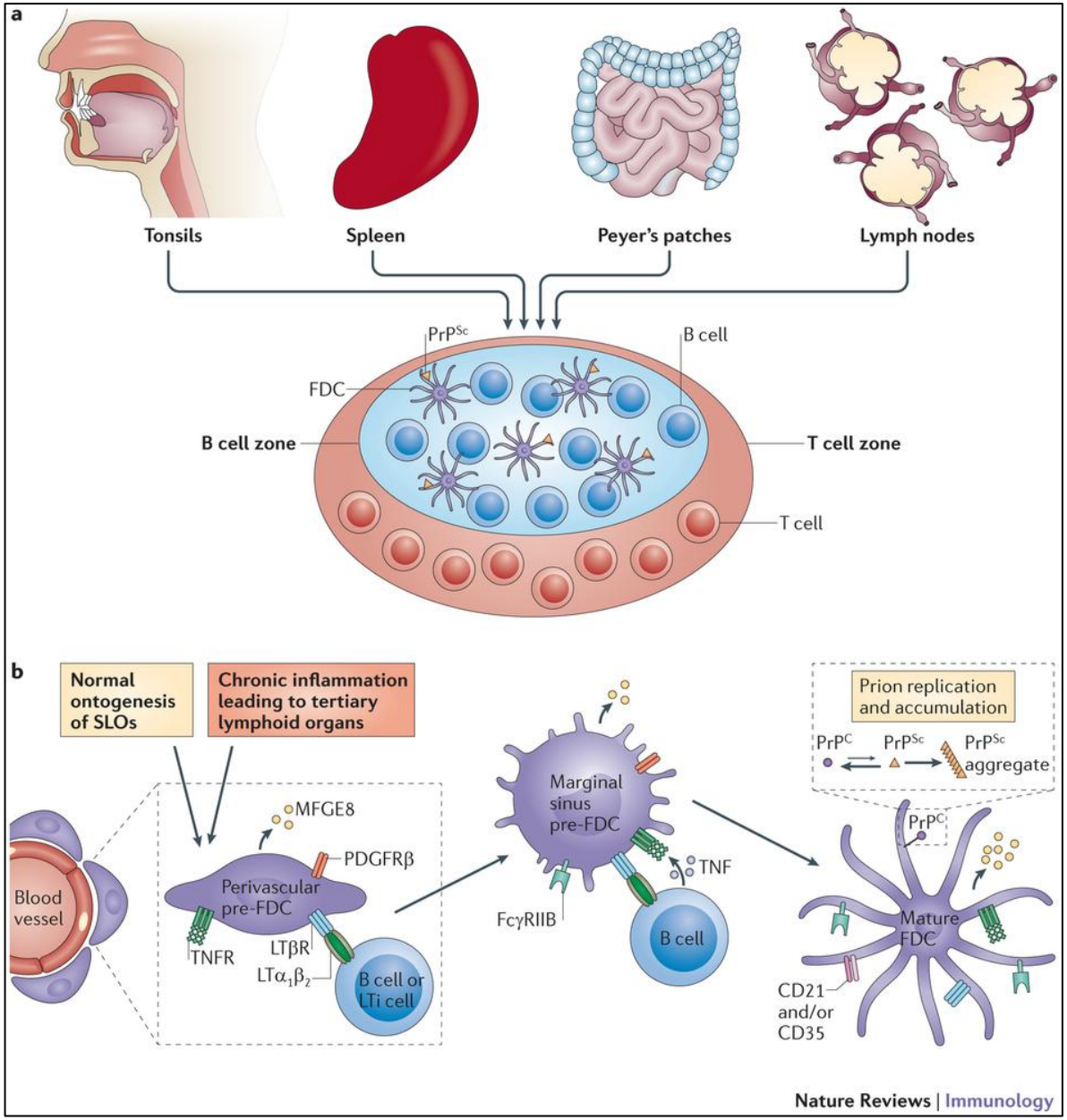
http://www.prion.ucl.ac.uk/research/mrc-research-groups/human-genetics/



The solid arrows indicate the recognized routes of prion transmission. Cases of iatrogenic Creutzfeldt–Jakob disease (iCJD) have occurred through corneal and dura mater transplantations from diseased cadaveric donors, through the use of prion-contaminated electroencephalography electrodes and neurosurgical instruments, and through the intramuscular administration of contaminated pituitary-derived hormones. Ingestion of meat from cows with bovine spongiform encephalopathy and cannibalistic rituals cause variant CJD (vCJD) and kuru, respectively. vCJD can also be transmitted through blood products. The dashed arrows indicate potential routes of prion transmission that have been suggested on the basis of experimental studies in animal models; their clinical relevance is currently unknown (Aguzzi et al., 2013).



The cascade of prion entry, peripheral replication, neuroinvasion, and neurodegeneration. After peripheral exposure, prions colonize and replicate in secondary lymphoid organs (SLOs) like spleen, Payer's patches, lymph nodes, and tonsils. FDCs are the main sites accumulating prions in SLOs. B cell-derived LTs and TNF facilitate prion accumulation by supporting development and maintenance of FDCs. Dedifferentiation of FDCs by LTβR-Ig delays neuroinvasion, whereas repetitive immunization accelerates prion pathogenesis. Prions reach the central nervous system (CNS) through autonomic nerves, directly after intracerebral inoculation, or via aerosols through immune-independent pathways. In the brain, prions replicate but are also cleared by microglia after opsonisation by astrocyte-borne Mfge8. Prion deposition comes about when PrP^{Sc} production exceeds PrP^{Sc} clearance (Aguzzi and Zhu, 2012).



a | Peripherally acquired prions replicate in lymphoid follicles of secondary lymphoid organs (SLOs; such as tonsils, spleen, Peyer's patches in the intestines and lymph nodes) and are mainly associated with follicular dendritic cells (FDCs). **b** | During normal ontogenesis of SLOs, FDCs emerge from platelet-derived growth factor receptor-β (PDGFRβ)-expressing ubiquitous perivascular pre-FDCs through an intermediate cell termed the marginal sinus pre-FDC. FDC maturation requires exposure to B cell- or lymphoid tissue inducer (LTi) cell-derived lymphotoxin-αβ heterotrimers ($LT\alpha_1\beta_2$) for the first transition from the perivascular pro-FDC stage to the marginal sinus pre-FDC stage, and exposure to B cell-derived $LT\alpha_1\beta_2$ and tumour necrosis factor (TNF) for the second transition from the marginal sinus pre-FDC stage to the mature FDC. This process is accompanied by upregulation and downregulation of numerous transcripts. Similarly, during chronic lymphocytic inflammation, perivascular pre-FDCs can differentiate into mature FDCs, thereby favouring the formation of tertiary lymphoid organs (TLOs), potentially at any site of the body. Mature FDCs express high levels of cellular prion protein (PrP^C) and are involved in peripheral prion replication and accumulation. FcγRIIB, low affinity immunoglobulin-γ Fc region receptor II-B; MFGE8, milk fat globule epidermal growth factor 8; LTβR, LTβ receptor; PrP^{Sc}, scrapie prion protein; TNFR, TNF receptor. (Aguzzi et al., 2013)

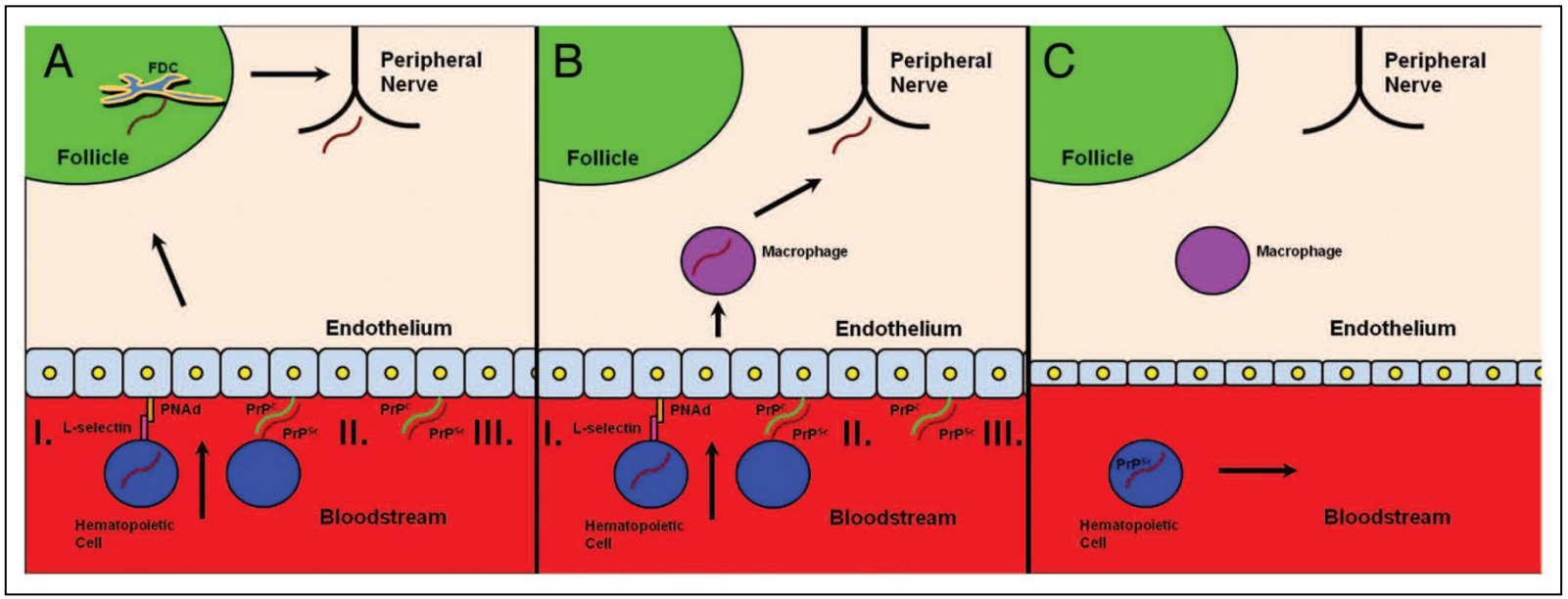


Figure 3. Possible mechanisms of TNFR1- and LTβR-controlled prion entry into lymph nodes. **(A)** Under normal conditions prions in the bloodstream are delivered to lymph nodes within hematopoietic cells (scenarios I and II) or as naked prions (scenario III). Prion-containing hematopoietic cells are taken up in HEVs by virtue of the classical PNAd:L-selectin interaction between lymphocytes and HEV endothelial cells (scenario I). Alternatively, prions may utilize a direct interaction between PrPC expressed on HEV endothelial cells and PrP^{Sc}, either free-floating (scenario III) or tethered to a hematopoietic cell membrane (scenario II). Once inside lymph nodes, prions are transported to the B cell follicles, where they are taken up by FDCs, replicated and finally transferred to peripheral nerves. **(B)** In the absence of TNFR1 signaling, the uptake of prions into lymph nodes is unaffected, but mature FDCs are de-differentiated. In the absence of mature FDCs, prions are engulfed by macrophages where they are degraded. However, at high enough titers, the number of prion molecules overwhelms the capacity of macrophages to efficiently degrade them, and prions accumulate and are transferred to peripheral nerves regardless of the fact that FDCs are absent. **(C)** In the absence of LTβR signaling, both FDCs and HEVs are de-differentiated. De-differentiated HEVs downregulate the expression of cell surface adhesion molecules (e.g., PNAd or PrP^{C}), which are required for the uptake of lymphocytes and/or prions into lymph nodes. Hence, the absence of FDCs in lymph nodes is inconsequential in this case, as prions can no longer enter lymph nodes or access the B cell follicles. HEV = high endothelial venules; (O'Connor and Aguzzi, 2013).

Animal prion diseases

Disease	Host	Etiology	Year of Description
Scrapie	Sheep, Goats	Infection with Prions of unknown origin	Mid 18th century
TME	Mink	Infection with Prions of either sheep or cattle origin	1947
CWD	Cervids	Infection with Prions of unknown origin	1967
BSE	Cattle	Infection with Prions of unknown origin	1986
FSE	Cats	Infection with Prions of BSE origin	1990

(Jeongmin lee et al.,2013)

Human prion disease

Sporadically

1-CJD

2-FATAL INSOMIA

3- variably protease-sensitive prionopathy

Hereditary

1-Familial CJD

2- Fatal familial insomnia

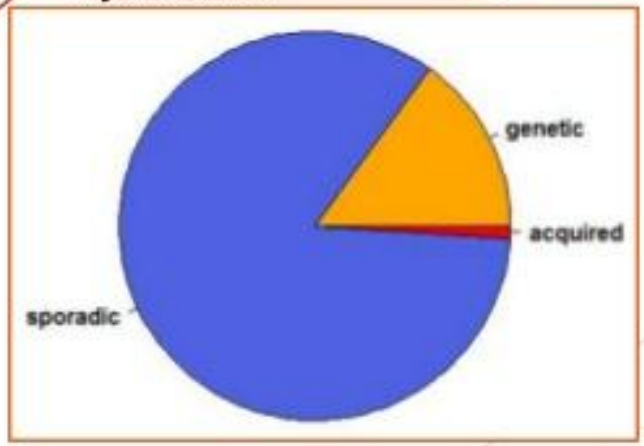
3- Gerstmann-Sträussler-Scheinker syndrome

Acquired

1-Kuru

2-Iatrogenic CJD

3-Variant form of CJD .



(Bucelli RC *et al.*,2013

Creutzfeldt-Jakob disease (CJD)

Most common human prionic disease. Rare disease

85-90% sporadic, incidence: 1/1.000.000 , peak VII decade

familial forms, associated with rare *PRNP* (PrP^{c}) mutations

Acquired forms cornea transplantation, contaminated GH preparations

Clinical onset: memory and behavior alterations, progressive dementia, involuntary muscle contractions, death within six month

Creutzfeldt-Jakob disease variant (vCJD)

1995, UK, several CJD-like cases

young individuals, early behavioral alterations, progression slower than classic CJD

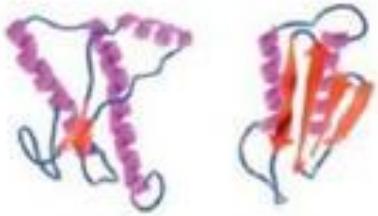
wide cortical plaques surrounded by spongiform alterations, but no mutated PrP

associated with bovine spongiform encephalopathy (BSE)

How Creutzfeldt-Jakob disease works

CAUSE

Creutzfeldt-Jakob disease is caused by abnormal proteins called prions that are not killed by standard methods for sterilizing surgical equipment.



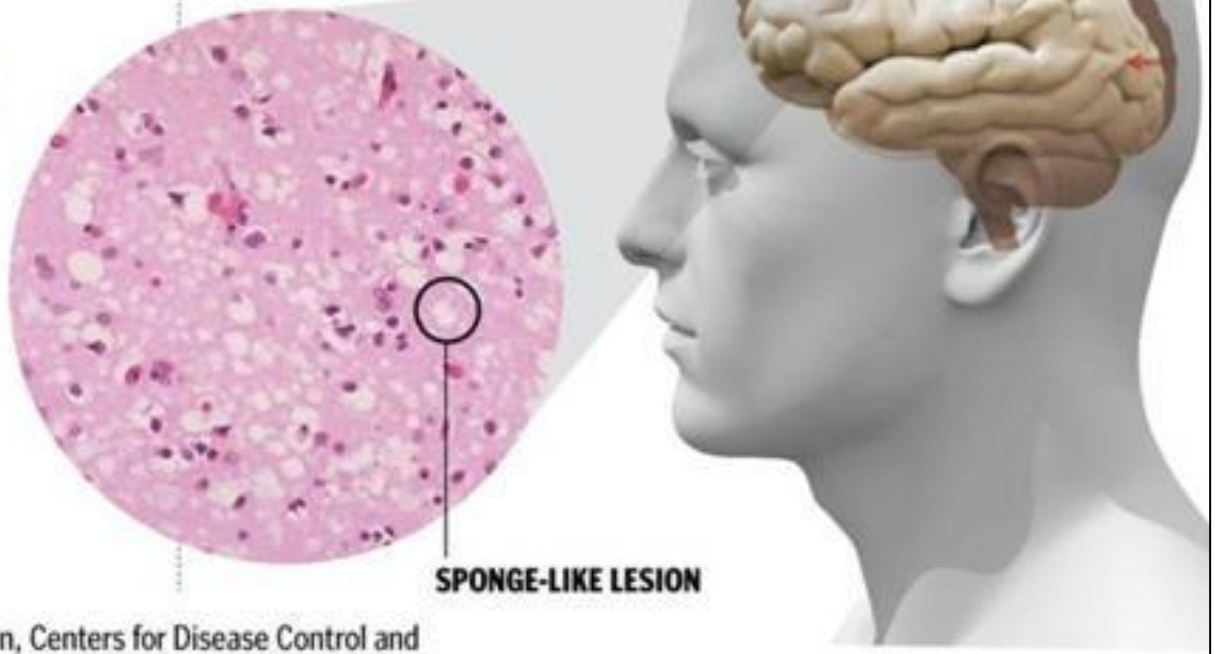
NORMAL HUMAN PROTEIN

DISEASE-CAUSING PRION

As prions build up in cells, the brain slowly shrinks and the tissue fills with holes until it resembles a sponge.

CONSEQUENCES

Those affected lose the ability to think and to move properly and suffer from memory loss. It is always fatal, usually within one year of onset of illness.

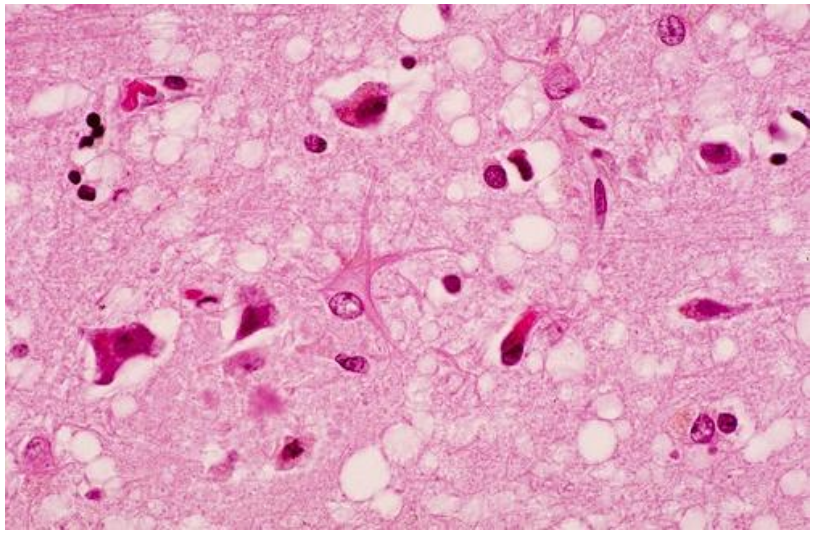


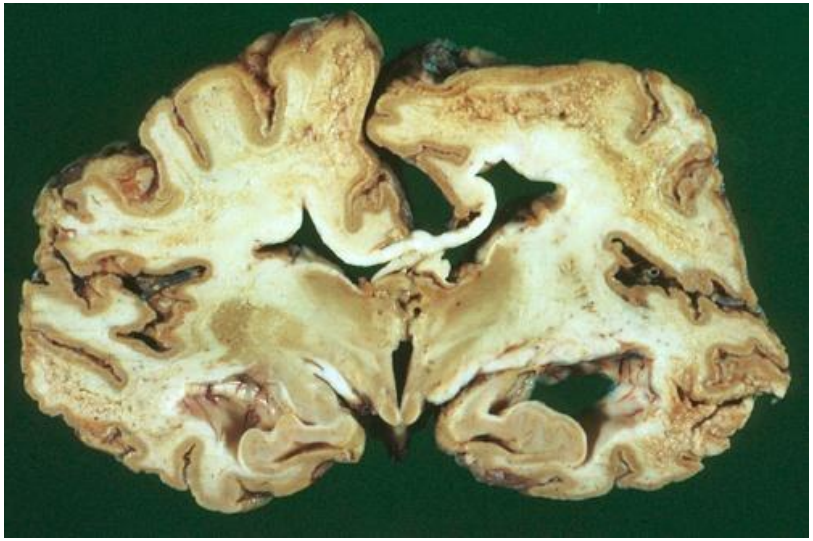
SOURCES: World Health Organization, Centers for Disease Control and Prevention, National Institute of Neurological Disorders and Stroke, AP

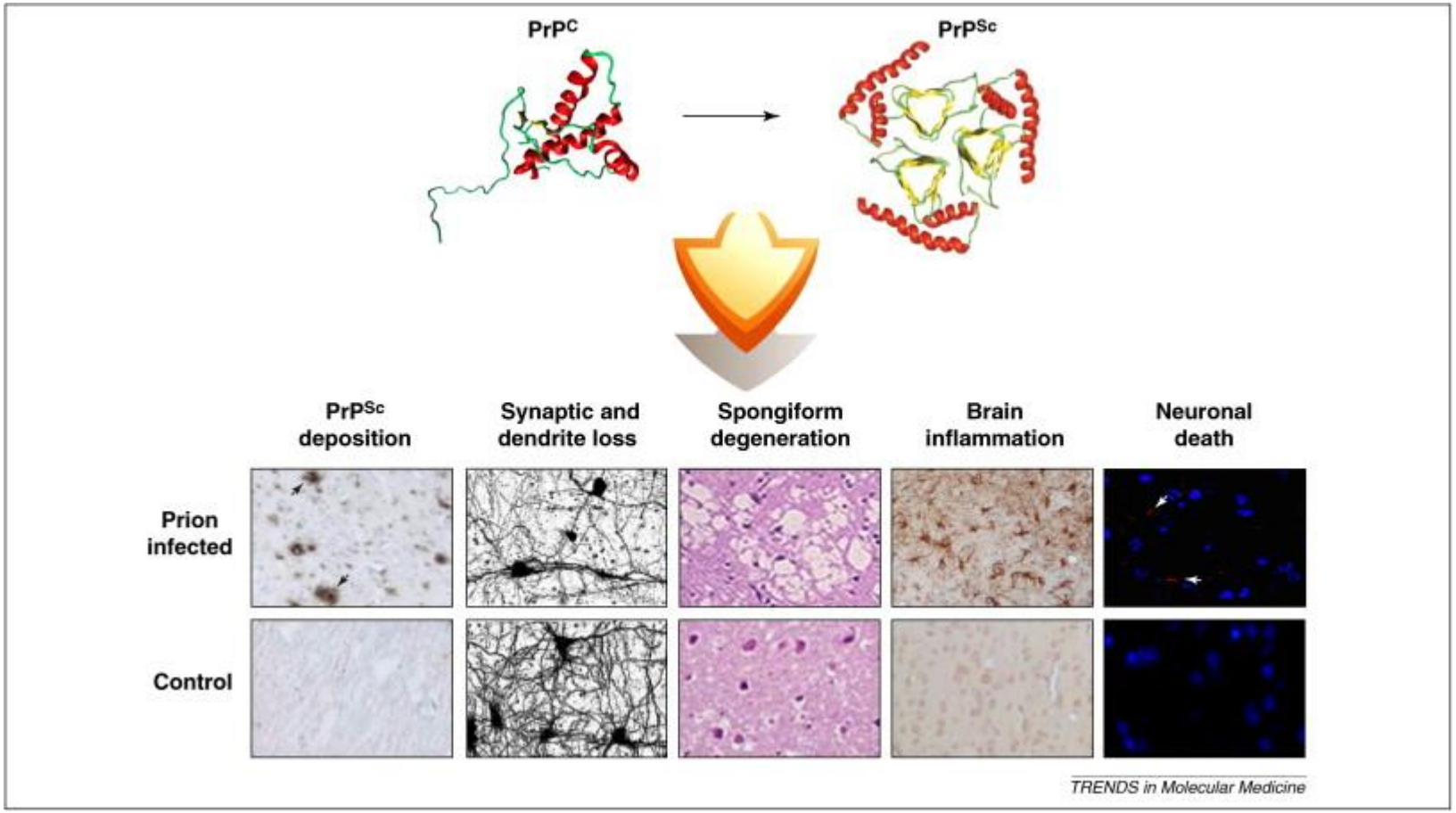
DAVID BUTLER, CHIQUI ESTEBAN, JAVIER ZARRACINA/GLOBE STAFF

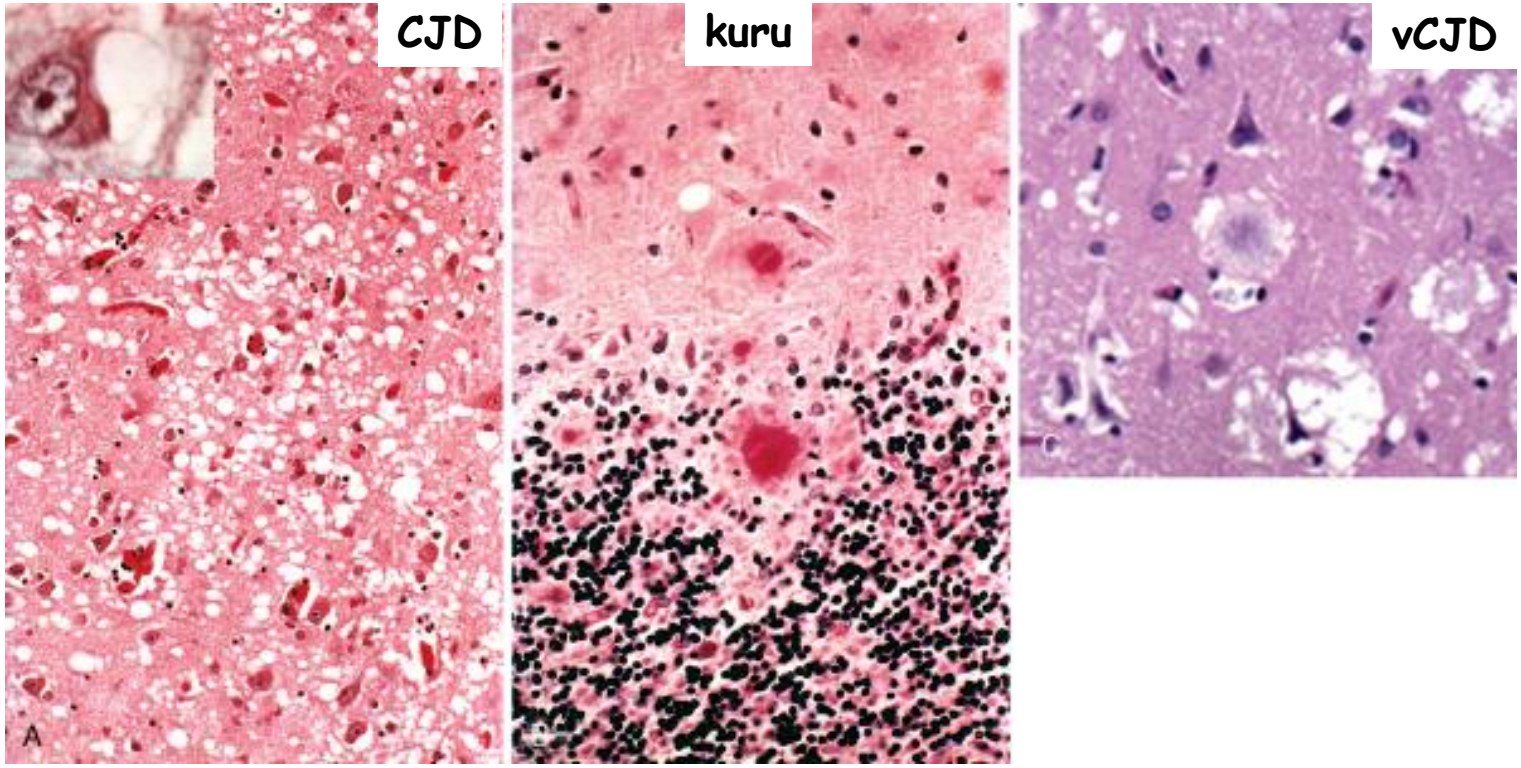
Histopathology

- spongiform vacuolization
- astrocytosis
- PrP-containing amyloid plaques
- neuronal loss



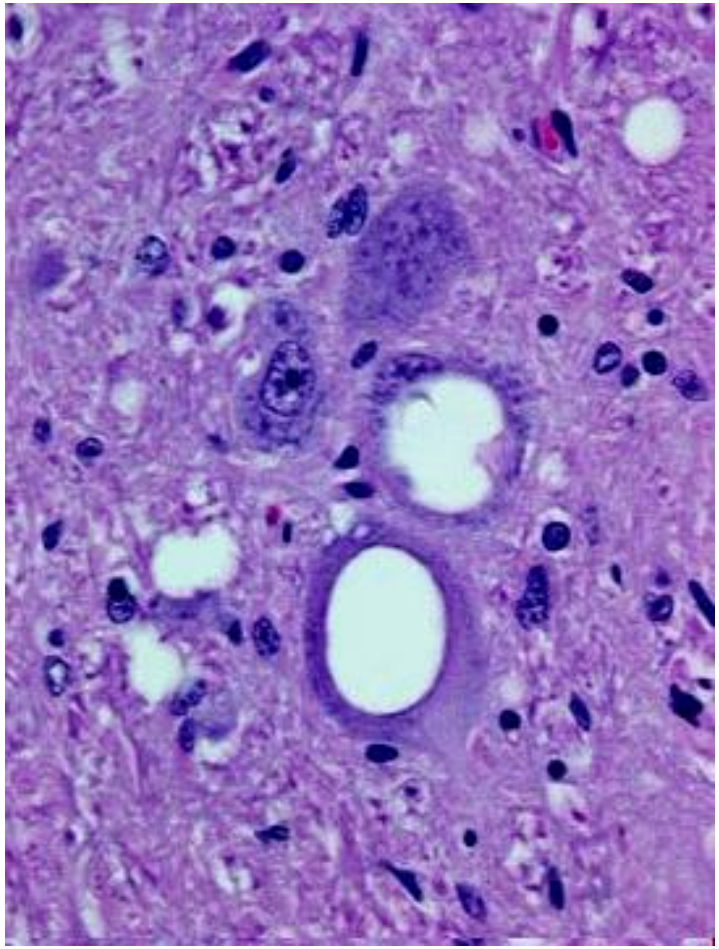


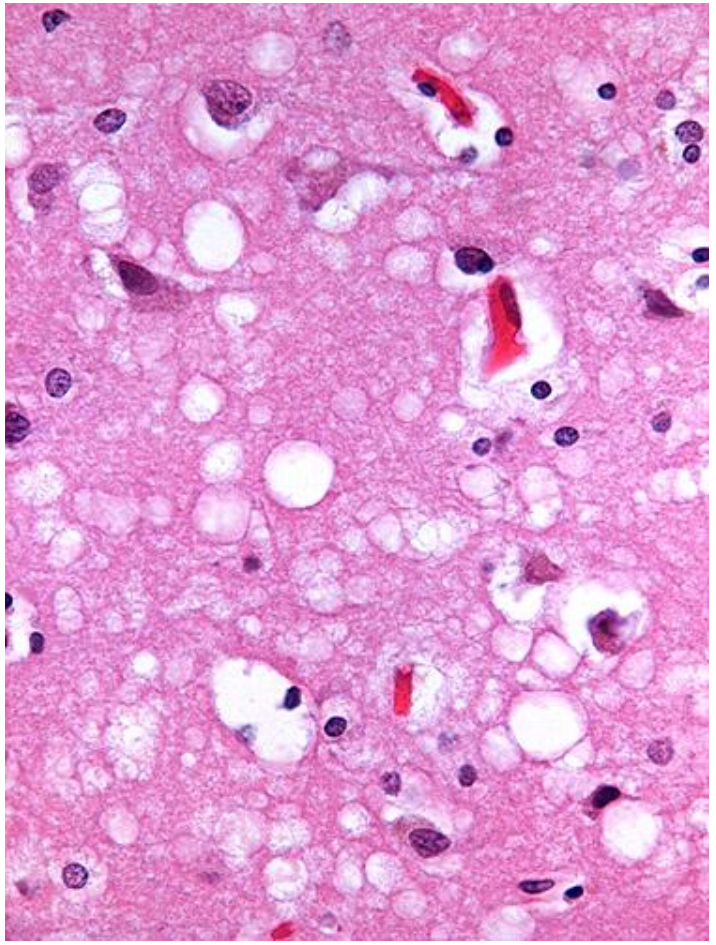

PrP^C
PrP^Sc
PrP^Sc deposition
Synaptic and dendrite loss
Spongiform degeneration
Brain inflammation
Neuronal death
Prion infected
Control
TRENDS in Molecular Medicine



CJD: cerebral cortex, spongiform aspect. Inset: vacuolized neurons
Kuru: cerebellar cortex with extracellular plaques of PrP^{sc} aggergates (PAS staining)
vCJD: cortical plaques surrounded by spongiform alterations

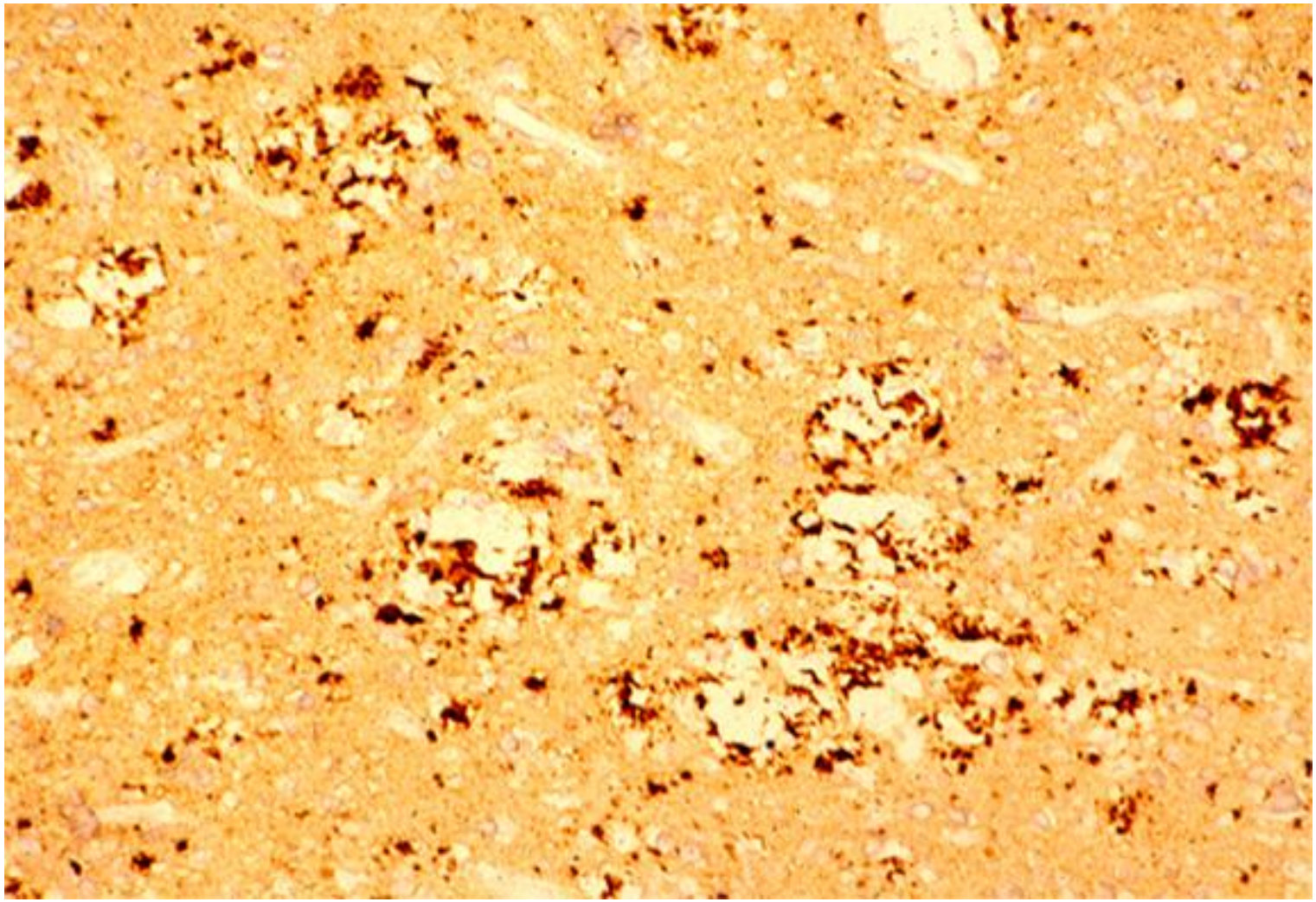
Scrapie sheep brain



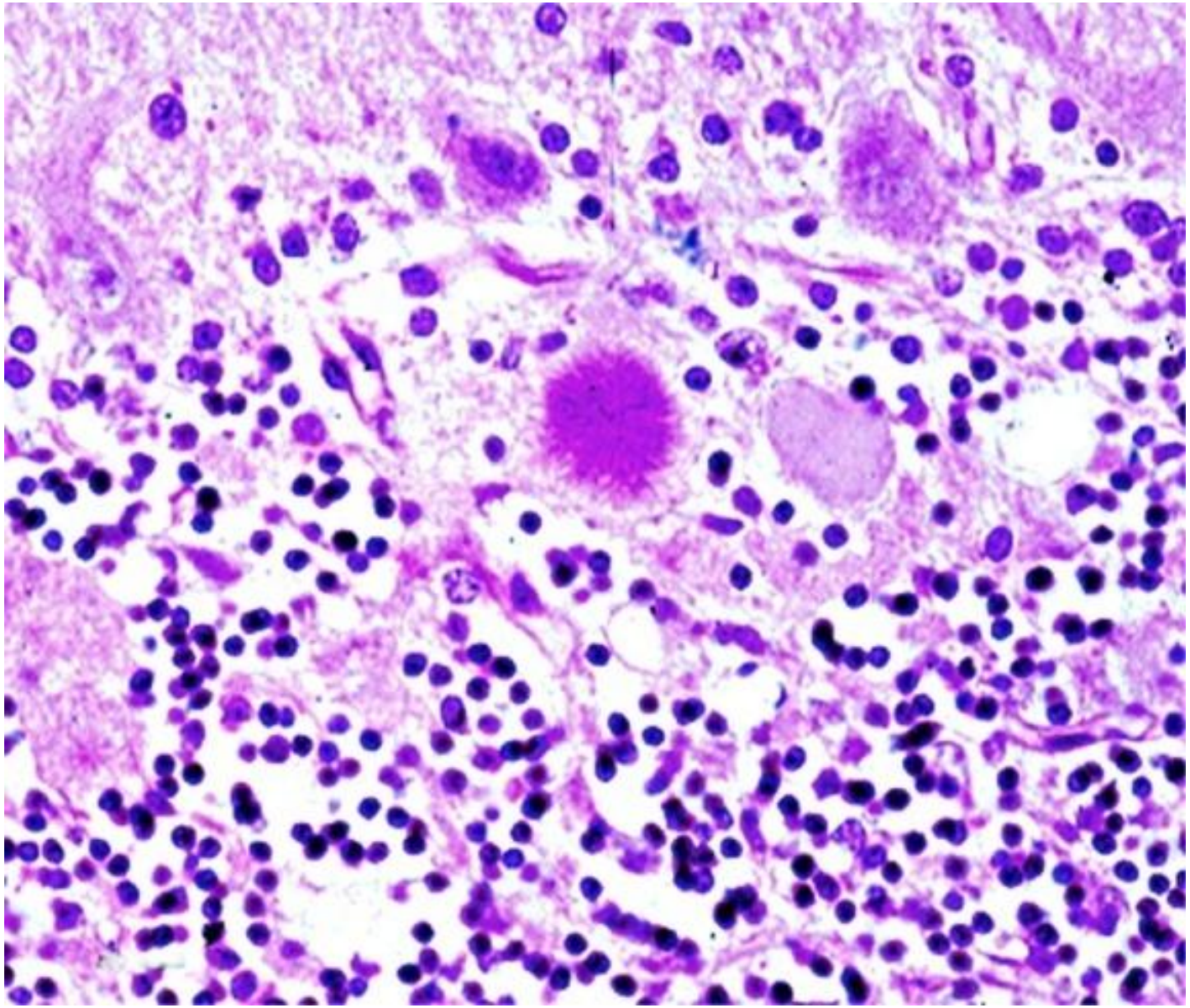


The same changes are seen in all of the spongiform encephalopathies, including BSE in cattle and CJD in humans. Large empty spots, called vacuoles, can be seen in two neurons. They are actually located within the cytoplasm of the cells, pushing the nucleus and the normal contents of the cytoplasm to the margins of the cells. It is these cavities that make infected brains seem to resemble a sponge, hence the name "spongiform".

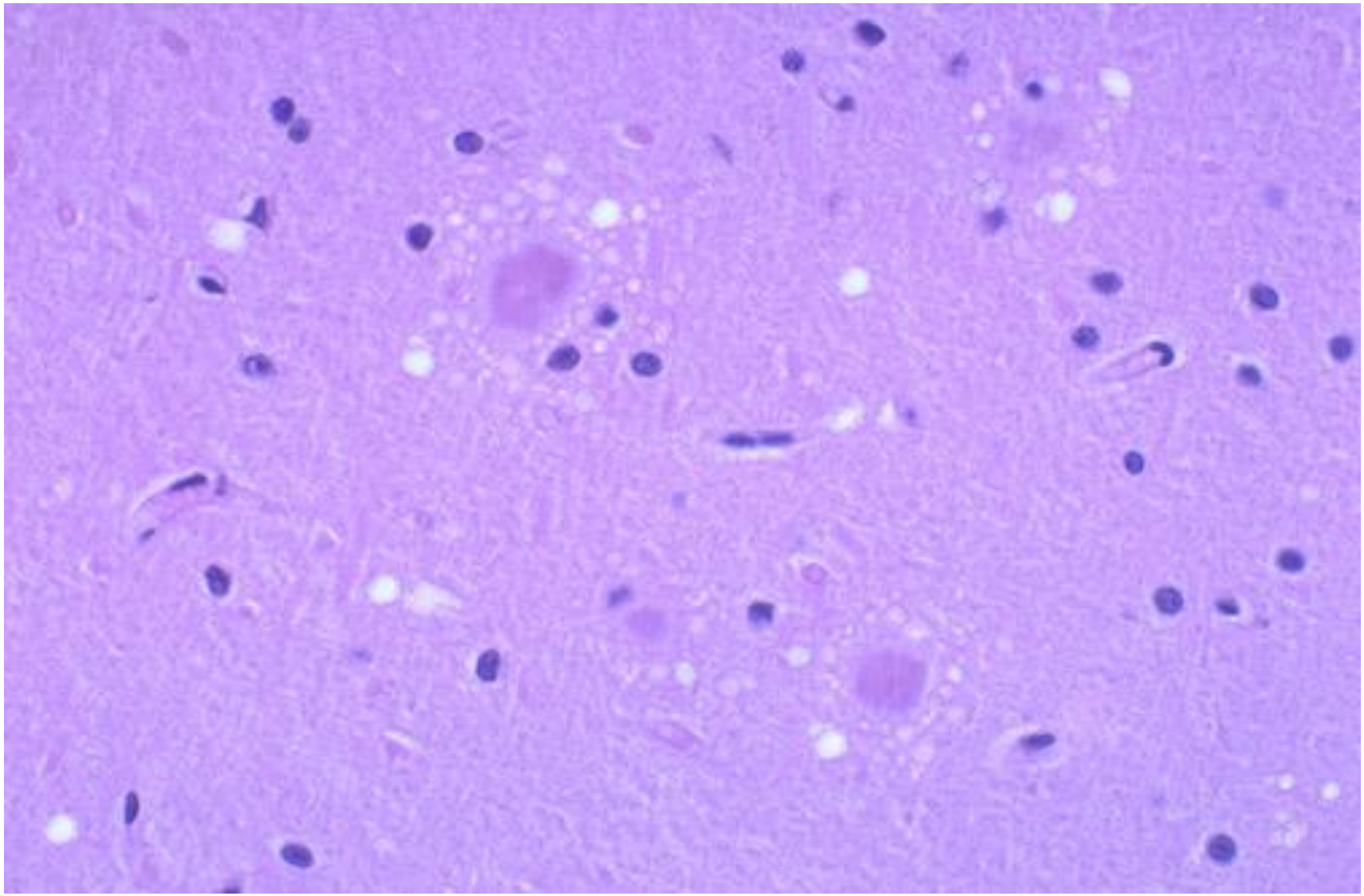
CJD The majority of the sections taken from the patient's cortex show this vacuolation that has been referred to as "spongiform degeneration". Most sections also reveal significant neuronal loss.



Immunoperoxidase stain using an **antibody that recognizes the prion protein in both its normal (PrP^{C}) and abnormal (PrP^{Sc}) forms.** The tissue was treated by hydrolytic autoclaving to denature the PrP^{C} but the PrP^{Sc} remains intact. So here the antibody is recognizing PrP^{Sc} around the vacuoles. The PrP^{Sc} can accumulate in amyloid plaques, but none are seen here.



Kuru plaque Section of cerebellum. The magenta-colored object in the center is called a Kuru plaque and it is made up of $PrP^{Sc/TSE}$



Note the rounded dark pink plaques, surrounded by prominent spongiform change, that are features of **vCJD**.

Incidence of vCJD cases reported worldwide