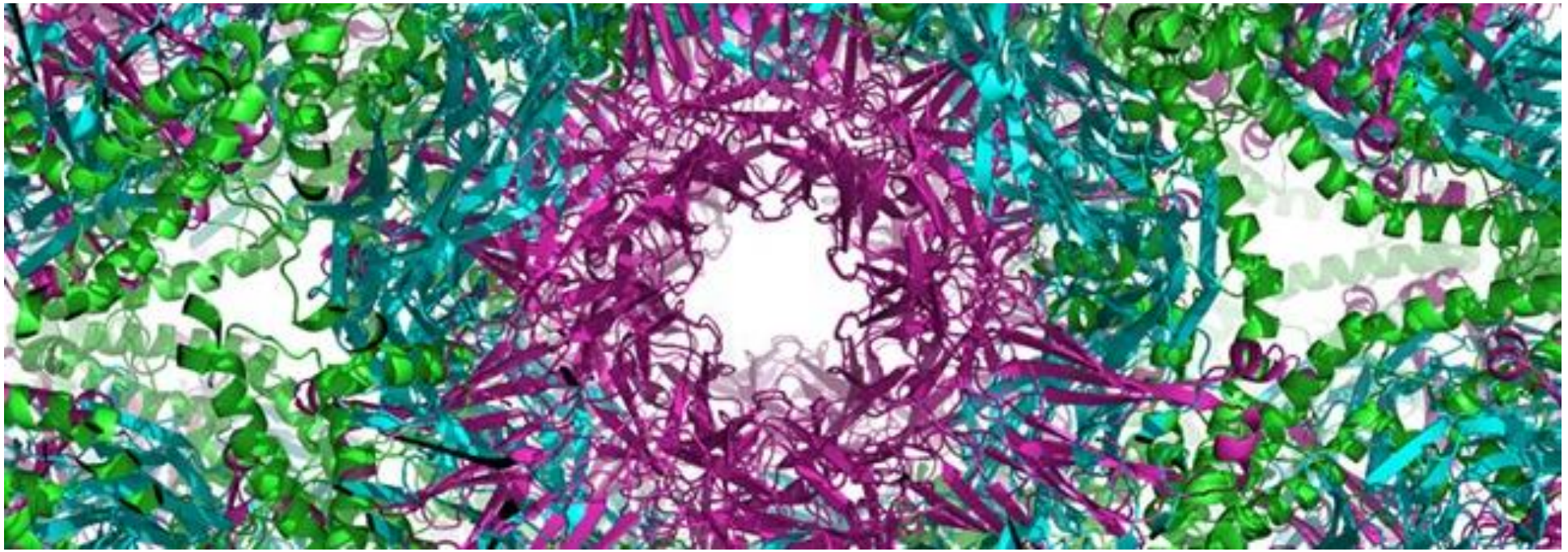
RUHR-UNIVERSITÄT BOCHUM

Prions and Security of Blood Products

A.Stang

Medizinische Fakultät
Abteilung für Molekulare und Medizinische Virologie



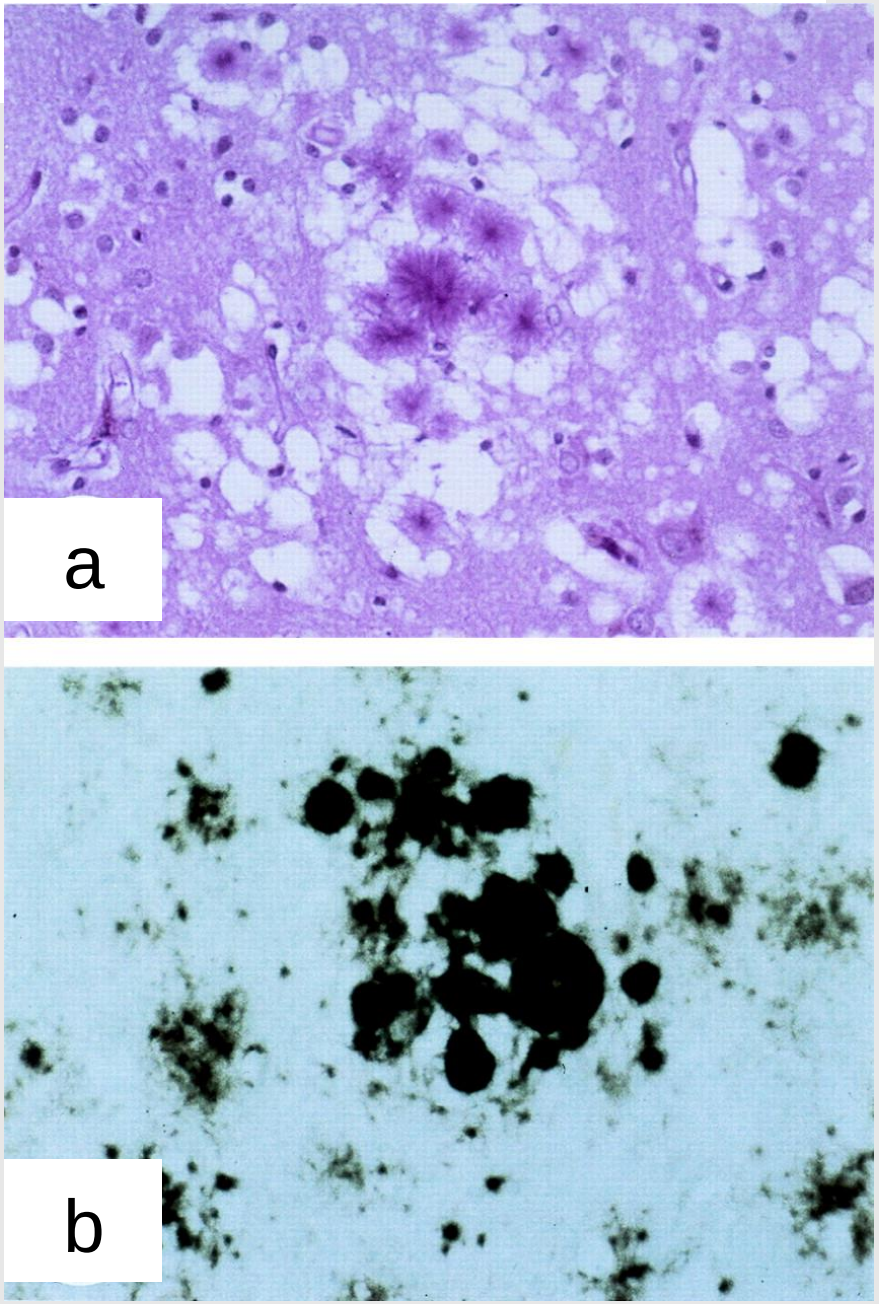
Übertragbare, spongiforme Enzephalopathien (TSE - transmissible spongiform encephalopathy)

- Chronic-progressive Infections of the CNS
- lethal
- Loss of Neurons (gaps) with Gliosis
- Amyloid plaques
- Diagnosis: post mortem by Pathologist
- In Humans: Creutzfeldt-Jakob-Disease (CJD)
- Sheep (Scrapie) known since more than 200 years

Histopathology of vCJD

Spongiform degeneration with central plaques

PrP-Immuno histology



Spongiform Enzephalopathies in animals

- Scrapie: (Sheep, Goat)
- BSE: Bovine spongiform encephalopathy (Cattle)
- TME: Transmissible mink encephalopathy (Nerz)
- CWD: Chronic wasting disease (Deers, Hirscharten)
- FSE: Feline spongiform encephalopathy (Cats)
- Exotic ungulate encephalopathy (var. zoo animals)

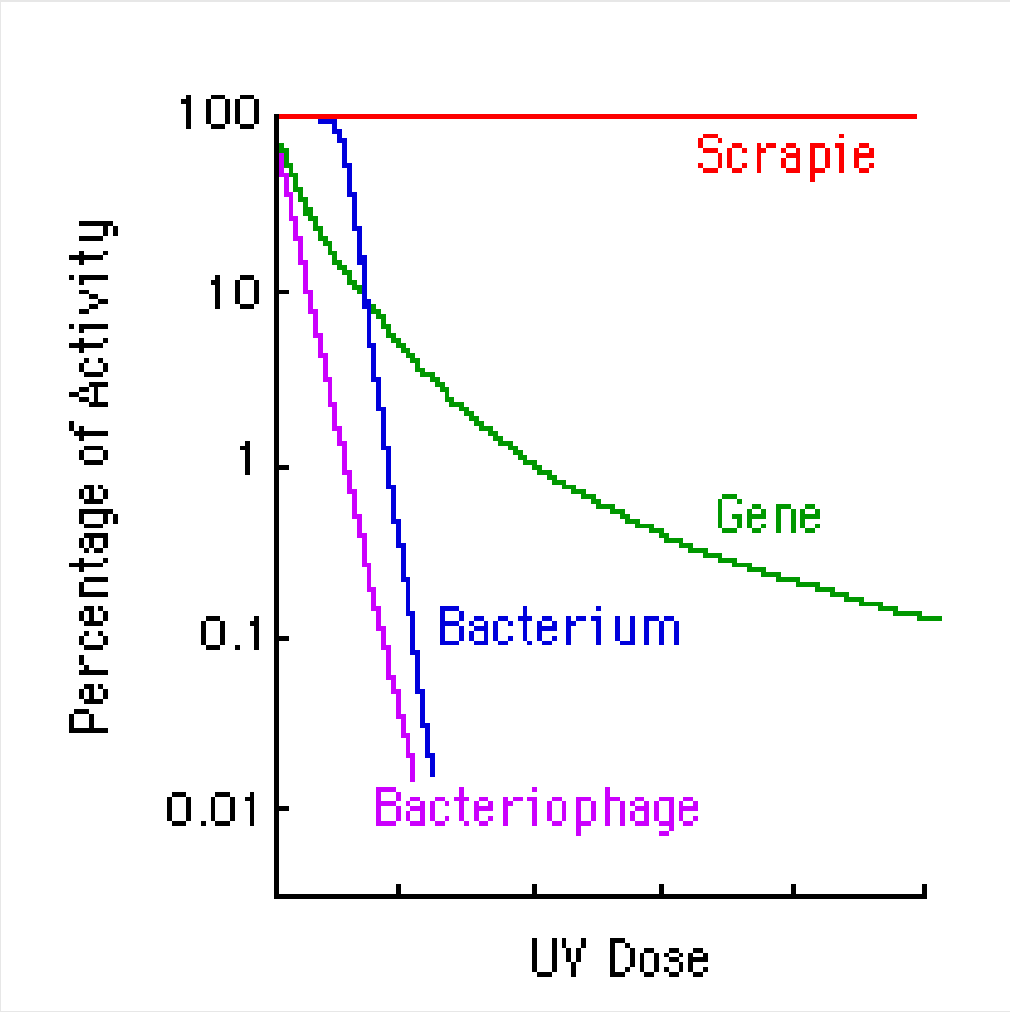
Scrapie (1752)

- In many parts of the world, probably started from Spain distributed in whole Europe and from Great Britain to the whole world
- Sheep and Goats: heavy neurologic symptoms, like scraping on fences (-> Scrapie)
- Incidence increases with age of animals; not younger than 1,5 years => Incubation period longer
- Lambs of diseased sheep more frequently diseased
- Pathogenic agent can survive at least 3 years in grass lands
- Detaild way of transmission unclear
- Pathogen primarily detectable in tonsills and mesenterial lymph nodes => oral transmission?

Infectious agent of Scrapie

- First a „slow-virus“- infection was assumed
- Extremely heat-stable
- Extreme ray and UV-resistance =>
- DNase and RNase resistant
- Sensitive to protein-denaturing agents (SDS, Phenol)

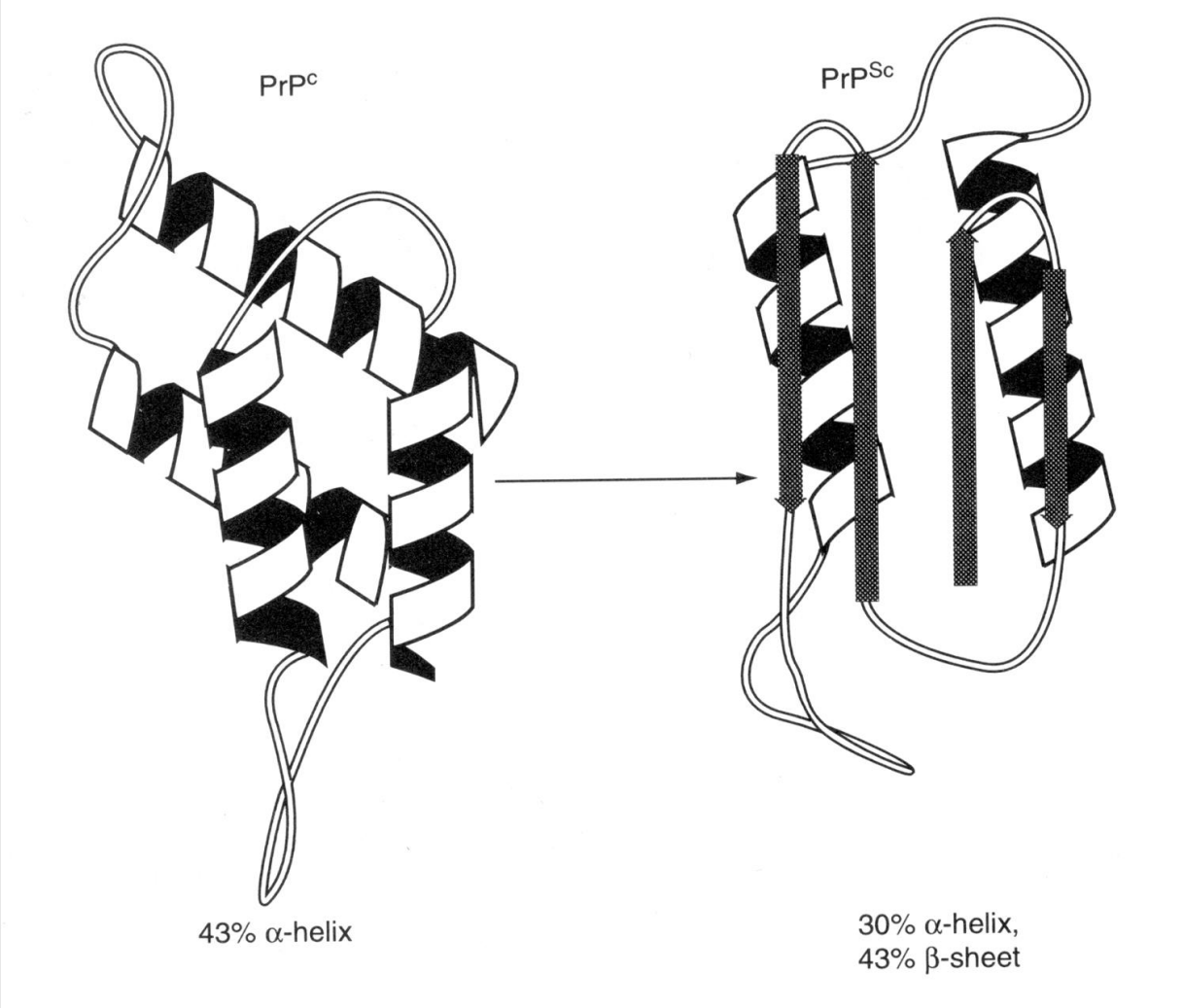
UV-Resistance of prions



The prion-concept

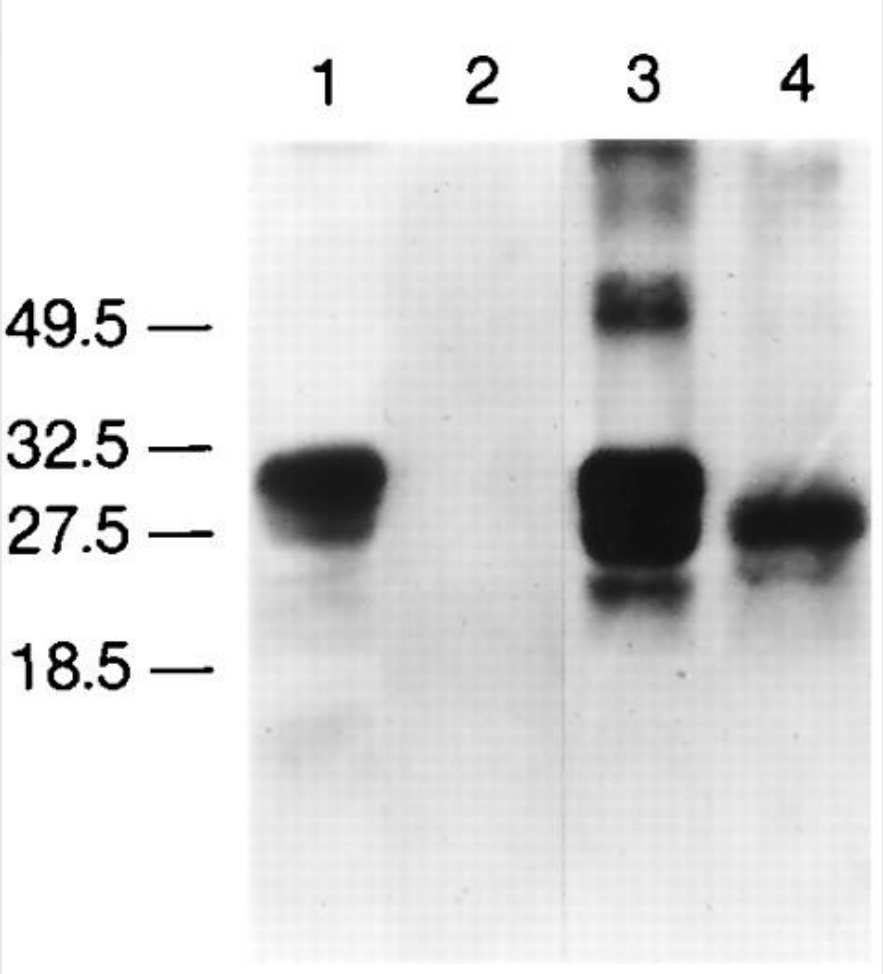
- (1982) Prusiner purifies a protein of 254 amino acids, which is associated with infection => PrP^{Sc}: Infectious protein?
- Identical amino acid sequence to cellular PrP^{C} protein: why pathogenic?
- Chemical identic
- Differnece in folding: PrP^{Sc} mostly beta-sheet structure
- Reason for partial resistance to Proteinase-K digestion => Detection assay for disease

Differences in secondary structure

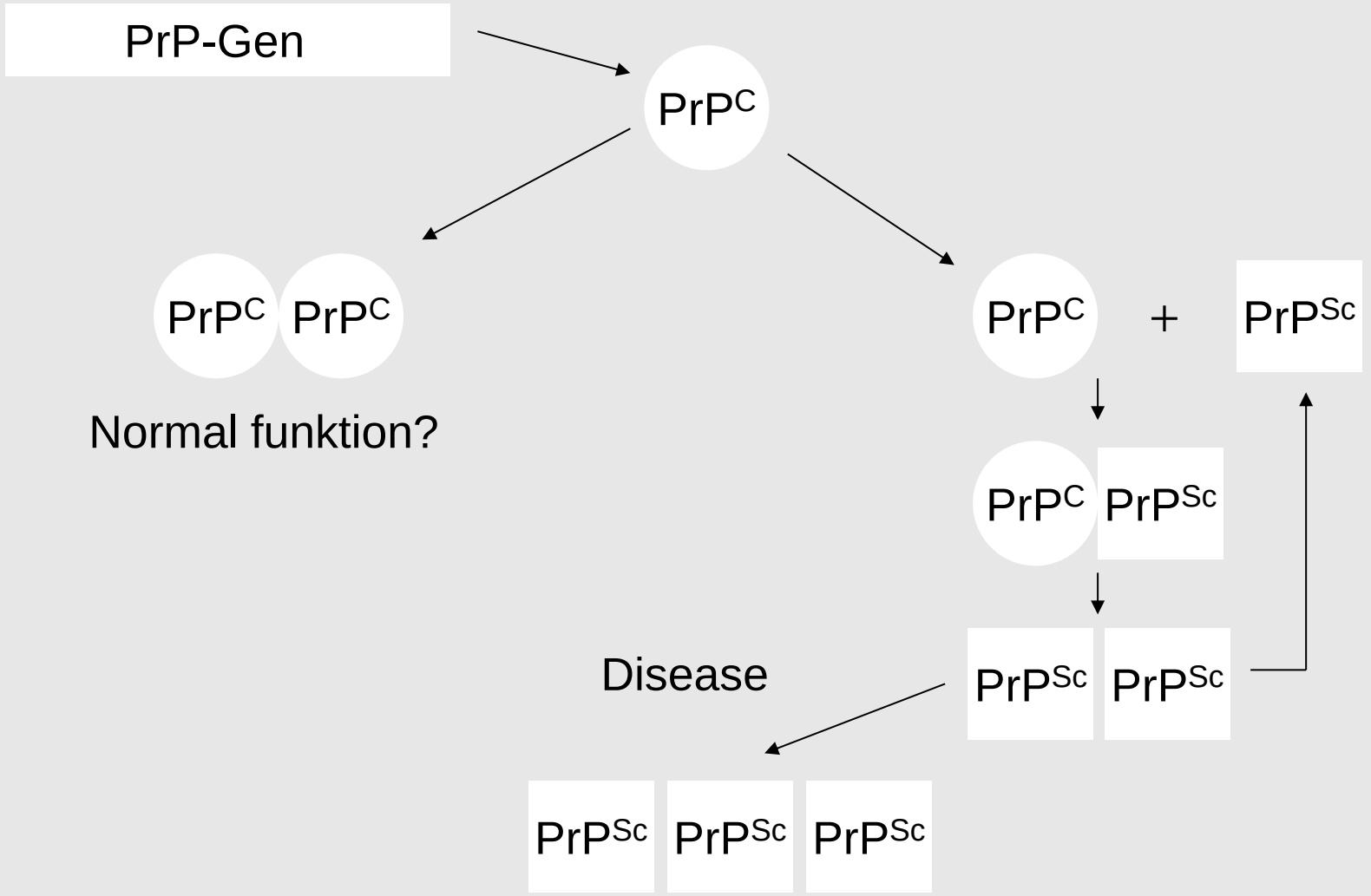


Immuno-Blot assay for detection of PrP^{Sc}

1: Normal brain extract
2: Normal brain extract + Prot. K
3: Brain extract infected
4: Brain extract infected + Prot. K

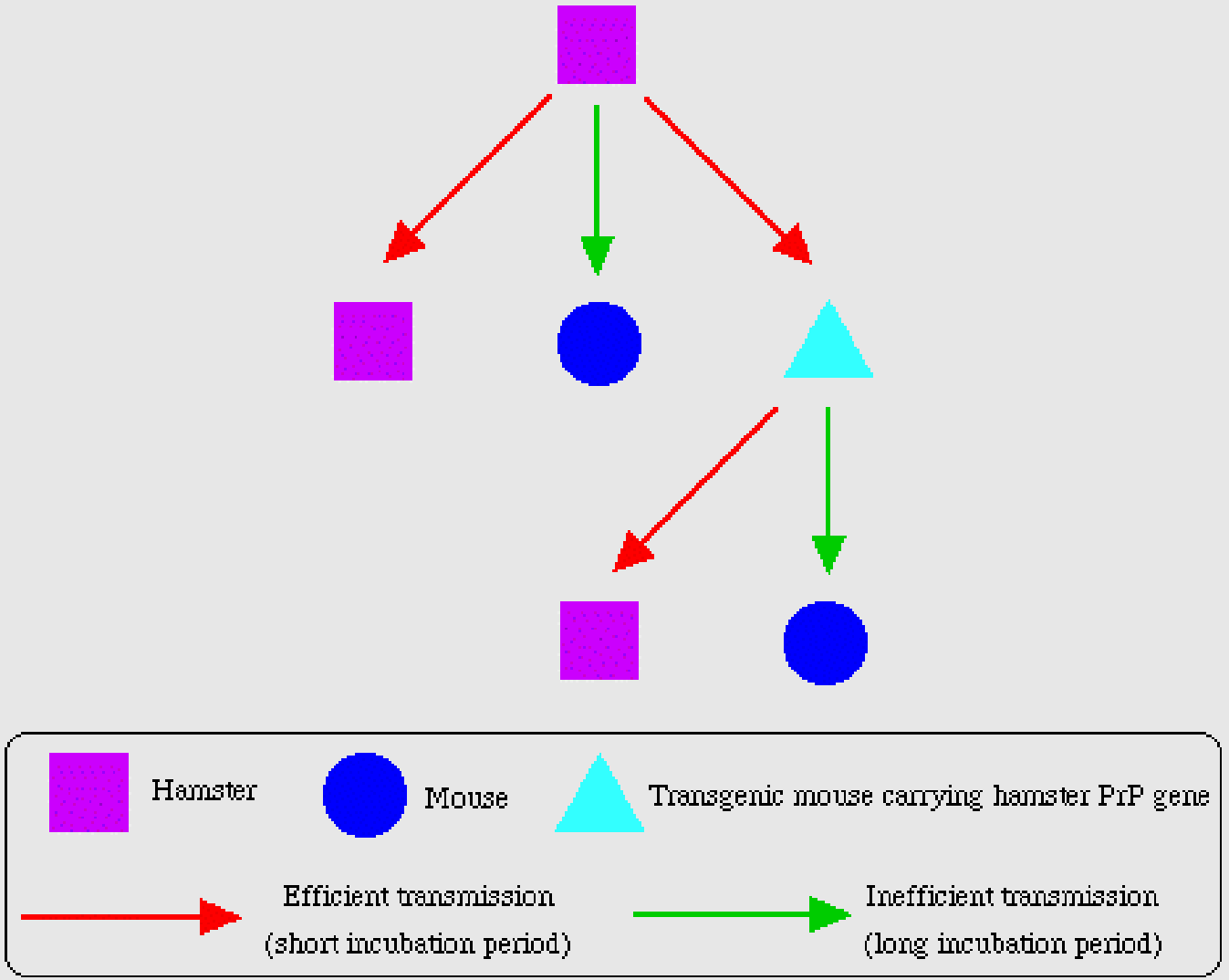


Relevance of PrP^C for TSEs



Experiments with transgenic mice

- PrP-Knock-out-mice do not develop TSEs
- Transmission of prions to other species: very long incubation times or no disease at all
- From second passage in new species transmission accelerated
- Characteristics of Prp^{Sc} can change with any passage => strain variations?


Hamster
Mouse
Transgenic mouse carrying hamster PrP gene
Efficient transmission
(short incubation period)
Inefficient transmission
(long incubation period)

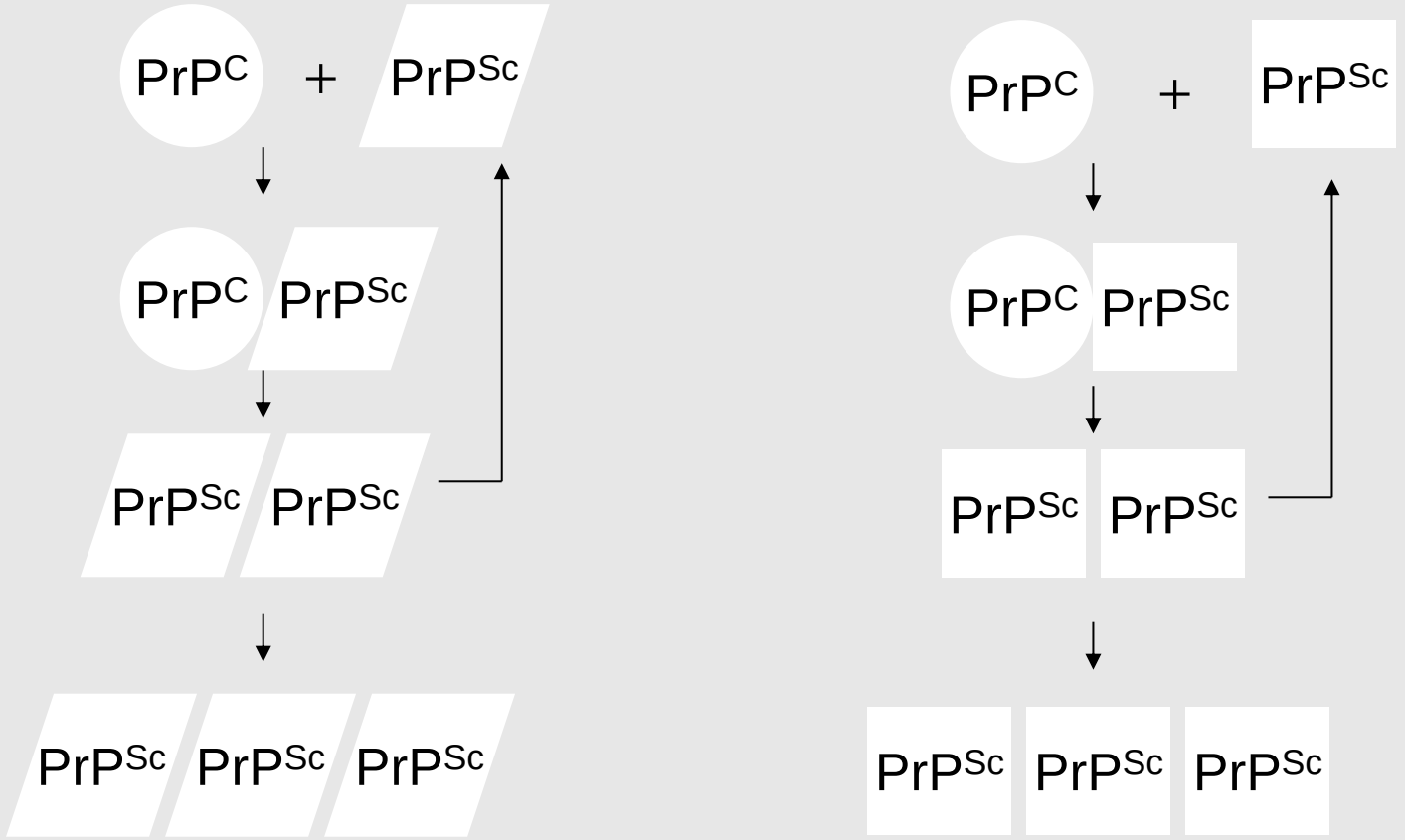
Strain-variations of prions

- Differences in incubation times and kind and distribution of lesions
- Reasons?

  - Additionally transmitted ligand of PrP^{Sc}?

  - Different versions of PrP^{Sc}?

PrP^{Sc} Strain-Hypothesis



Different courses of disease: More than 15 strains?

TSE´s in Humans

- Creutzfeldt-Jakob-Erkrankung (CJD)
- Lethal familial insomnia
- Gerstmann-Sträussler-Scheinker-Disease
- Kuru

Symptoms of CJD

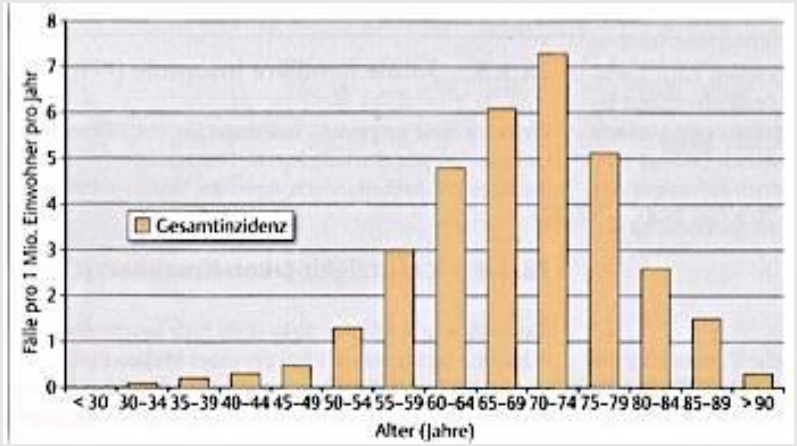
- Depression-like changes in personality, recognized only in retrospect
- Memory disorders are primarily explained by aging
- Visual impairments, walking instabilities or psychotic episodes lead to diagnosis, in most cases already 4 moths have passed
- Progressively myoclonic twitches
- Final state: Akinetic Mutism

Pattern of symptoms is defining, not single symptoms

Creutzfeldt-Jakob-Disease

Sporadic CJD:

- Incidence $1/10^6$
- Mean age: 65 years
- Lethal mostly within 3 months
- 90% of all TSEs in humans



- Cause: Somatic mutation in PrP-Gene or still unknown way of transmission of PrP^{Sc}?

Inherited Form:

- 10% of all TSEs in humans
- Autosomal-dominant inherited disease: Point mutation in PrP-Gene
- 20 different mutations

Creutzfeldt-Jakob-Erkrankung

Iatrogenic form:

- Neuro surgery
- Transplantations (Dura mater)
- Human growth hormones (hGH) from hypophyses of deceased people
- Reason: probably contamination with PrP^{Sc} from sporadic or inherited disease (confirmed by animal experiments)
- More than 490 cases up to now

=> CJD can be considered an infectious, familial disease

Iatrogenic transmission of CJD (Brown, 1999)

Übertragungsmodus	Anzahl Fälle	Eintrittspforte ins Gehirn	Mittlere Inkubationszeit (range)
Applikation von aus Leichen gewonnenem Wachstumshormon	>125	hämatogen	12 Jahre (5–30)
Applikation von Gonadotropin	5	hämatogen	13 Jahre (12–16)
Ungenügend sterilisierte Instrumente bei neurochirurgischen Operationen	5	intrazerebral	19 Monate (12–28)
EEG-Elektroden	2	intrazerebral	18 Monate (16–20)
Dura-mater-Transplantate	>106	Hirnoberfläche	6 Jahre (1,5–16)
Korneatransplantate	3	N. opticus	17, 18, 320 Monate

Inactivation of prions through chemical procedures

Partiell wirksam[1]	Nicht befriedigend bzw. ungenügend
• 1 N Na-Hydroxidlösg.[1], 1 h, 20 °C	• β-Propiolacton
• Na-Hypochloritlösg. mit 2 % freiem Chlor[1], 1 h, 20 °C	• Glutaraldehyd
• 1 N NaOH in Kombination mit 5 % SDS oder 1 N Hypochlorit plus autoklavieren (121 °C, 30 – 90 min)	• Wasserstoffperoxid
• 1 N NaOH (1 h) plus autoklavieren (121 °C, 30 – 90 min)	• Peressigsäure
• 4 M Gdn SCN (≥ 30 min)	• 2-Chlorethanol
• Kochen in 1 N NaOH	• 1 % Na-Hypochlorit (1 h)
	• Dnasen
	• Rnasen
	• Ethanol (70 oder 100 %)
	• Phenole
	• Tenside
	• Ammoniak
	• Proteinasen
	• Aceton
	• Diethylether
	• Chlordioxid
	• Säuren
	• K-Thiocyanat
	• Quartäre Ammoniumverbindungen
	• Formaldehyd (erhöht sogar die Stabilität)
	• Ethylenoxidsterilisation
Eine Inaktivierung ist ausschließlich durch proteindenaturierende Prozesse möglich	
Für wiederverwendbares Material bzw. Instrumente gilt: Einlegen in 5 % Hypochlorit, 1M NaOH oder NaOH/SDS (0,075 M/1 %) für 20 min (z. B. Metallinstrumente) bis mehrere Tage (z. B. Glaswaren) und anschließendes Autoklavieren (133 °C, 30 min)	

Gdn SCN - Guanidinisothiocyanat

Inactivation of prions through physical procedures

Ausreichend	Nicht befriedigend bzw. ungenügend
• 132 °C, 1 h Autoklav[1]	• UV-Bestrahlung
• 134–138 °C, 18 min für programm-gesteuerte Autoklaven[1] (mit Vorvakuum)	• Gammabestrahlung
• 121 °C, 4,5 h (Autoklav)	• 160 °C, 24 h bei trockener Hitze
• 135 °C, 1 h (Autoklav)	• 360 °C, 1 h bei trockener Hitze (lyophilisierte Erreger)
	• einfaches Kochen in Wasser

[1] empfohlen durch die WHO (1992)

Kuru

- First human TSE, which appeared endemically in some villages of New-Guinea
- Loss of neuronal control: Walking insecurities before Demencia, death within one year
- Affected: Grown-up women, sometimes juveniles, rarely men and children
- About 200 cases of 10.000 persons
- Cannabalistic funeral ritual of women and children
- At the end of 1950s rituals were not performed anymore: Strong reduction, new cases only at former participants
- Incubation time 3 – 35 years
- Probably transmission of sporadic PrP^{Sc} (around 1900)

Tödliche familiäre Insomnie - FFI (fatal familial insomnia)

- PrP_{178} Asp-Asn mutation
- Thalamic degeneration, cerebral spongiform changes variable, Loss of neuronal control: Death within one year
- Clinic: Insomnia dominant in the beginning
- Occurance mostly in the age of 37-61 years
- Atrophy of Thalamic Nuclei, Olivary Nuclei and Cerebellum

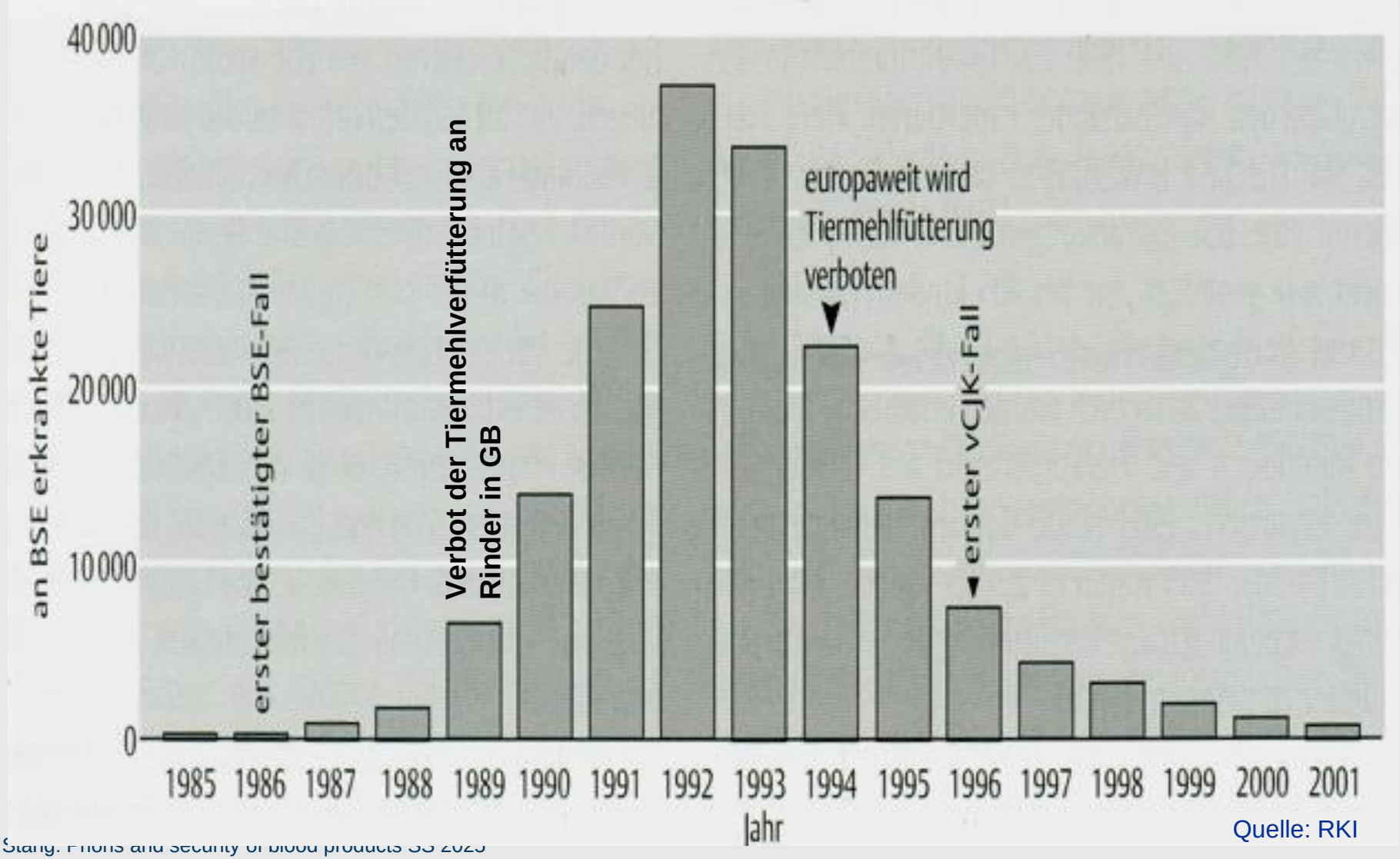
Gerstmann-Sträussler-Scheinker-Disease

- PrP_{102} Pro-Leu mutation
- Enzephalo-Myelopathy with multicentric PrP-Plaques
- Clinic: In the beginning Ataxia dominant symptom, after that demencia
- Incidence: 1:10 Mio.
- Occurence mostly at the age of 35-50 years
- Time of disease 2-6 years

BSE (bovine spongiform encephalopathy)

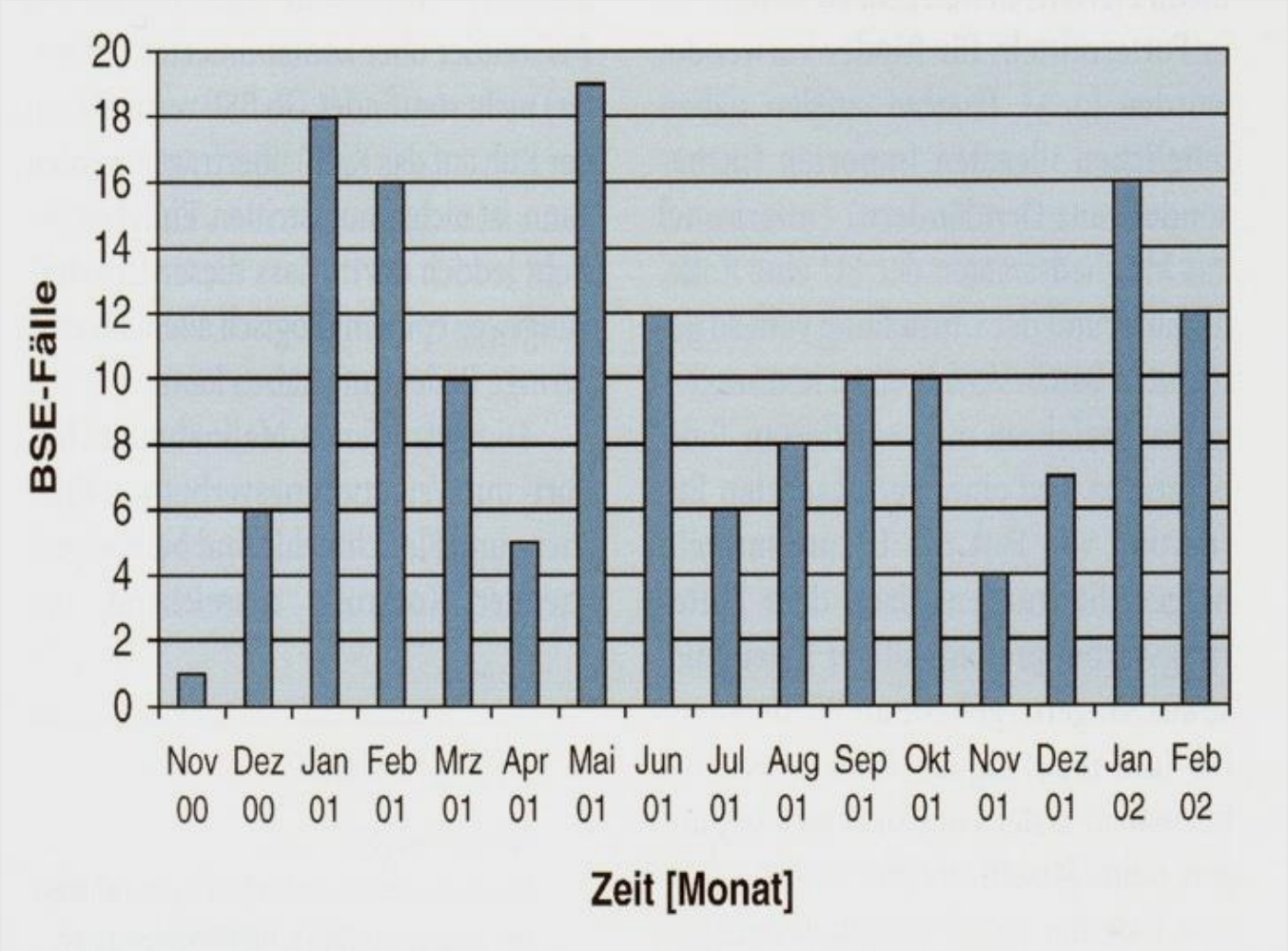
- 1986 first time identified in Great Britain
- 1996: 160.000 cases in GB 412 outside of GB documented
- Estimated number of unknown cases: Disease mostly occurs not before the age of 3 years, slaughtering already in the age of 1 to 2 years
- Changes in production of meat and bone meal: BSE infectious agent not inactivated completely anymore
- Where does BSE come from?
  - Probably not from sheep, due to different strain characteristics between PrP^{Sc} und PrP^{BSE}
  - Spontanous mutation, which could spread due to bad feeding habbits?
  - Other ways of transmission?

BSE-Epidemia in GB



Quelle: RKI

BSE-Epidemia in Germany



Quelle: RKI

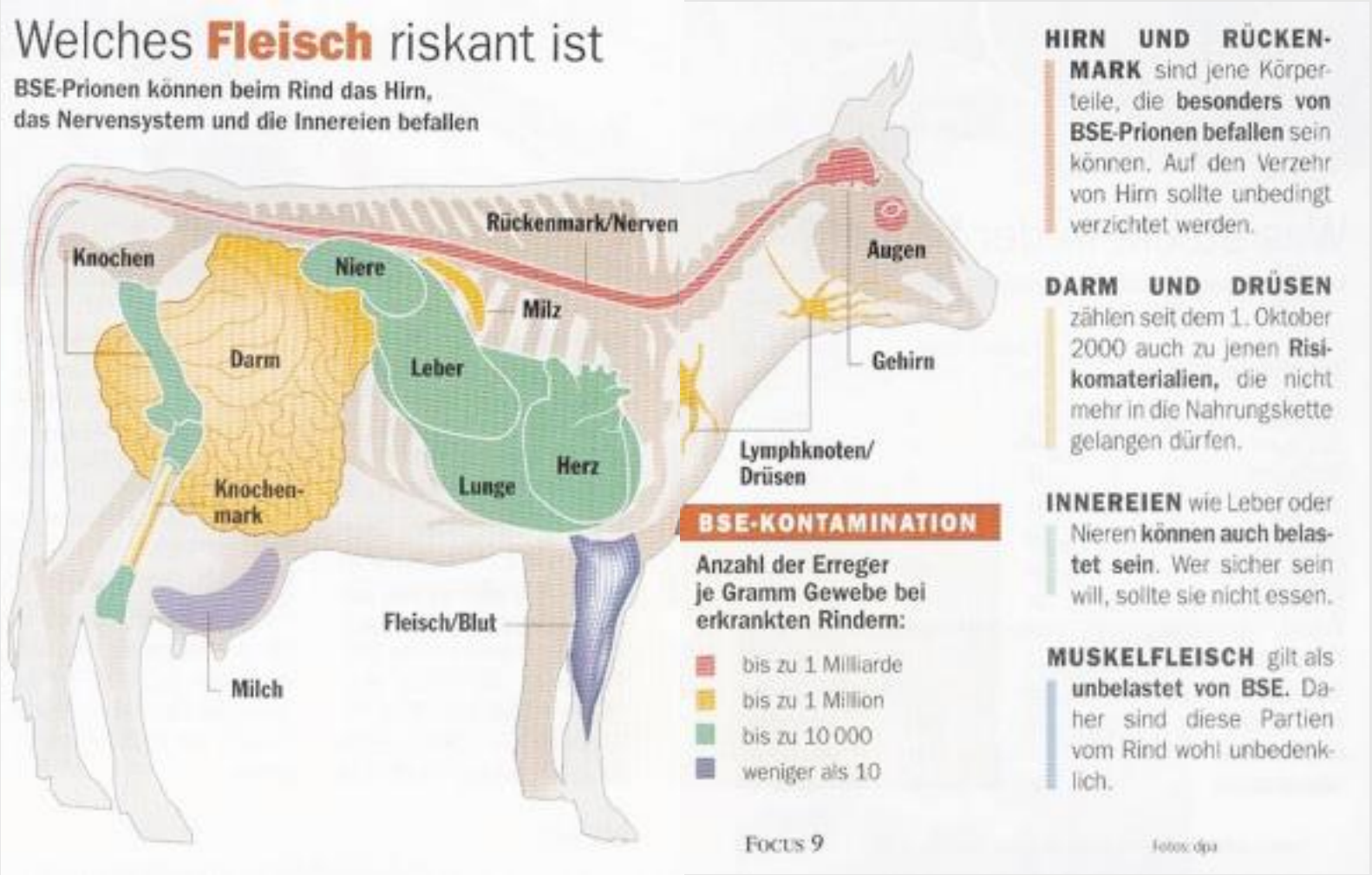
Transmission of BSE to humans

- 1996: 10 cases of CJD with unusual courses
- Mean age: 27 instead of 65
- Slower progression: 13 months instead of 3
- Cerebellar symptoms and heavy cognitive disorders
- Amyloid plaques like Kuru
- => vCJD (variant) up to today probably >160 cases

Special characteristics of new variant CJD

	sporadische CJK	vCJK
Typisches Alter bei Ausbruch	55 – 70 Jahre	13 – 56 Jahre (Median 27)
Erste Symptome	Demenz, Myoklonus	psychiatrische Auffälligkeiten (Angst, Depression, Zurückgezogenheit u. a.)
Verlauf	rasch voranschreitend	schleichend mit neurologischen Symptomen (zerebelläre Ataxie, Dysästhesien), später Demenz, Myoklonus
Elektroenzephalogramm	meist pathognomonisch	allgemeinverändert, nicht pathognomonisch
Überlebenszeit (Median)	4 – 6 Monate	13 Monate
PrP-Genotyp am Codon 129	vorwiegend homozygot (Val/Val oder Met/Met)	bislang stets methioninhomozygot (Met/Met)
PrP^{Sc}-Ablagerungen	synaptisch, selten Plaques	auffallende „floride" Plaques
PrP^{Sc}-Bandenmuster im Western-Blot	Typ 1 oder 2, bei iatrogenen Fällen mit intramuskulärer Inokulation Typ 3	Typ 4, ähnlich dem Muster von experimenteller BSE-Infektion bei Mäusen, Affen und anderen Arten

Distribution of infectivity



Animal experiments giving hints to transmission of BSE

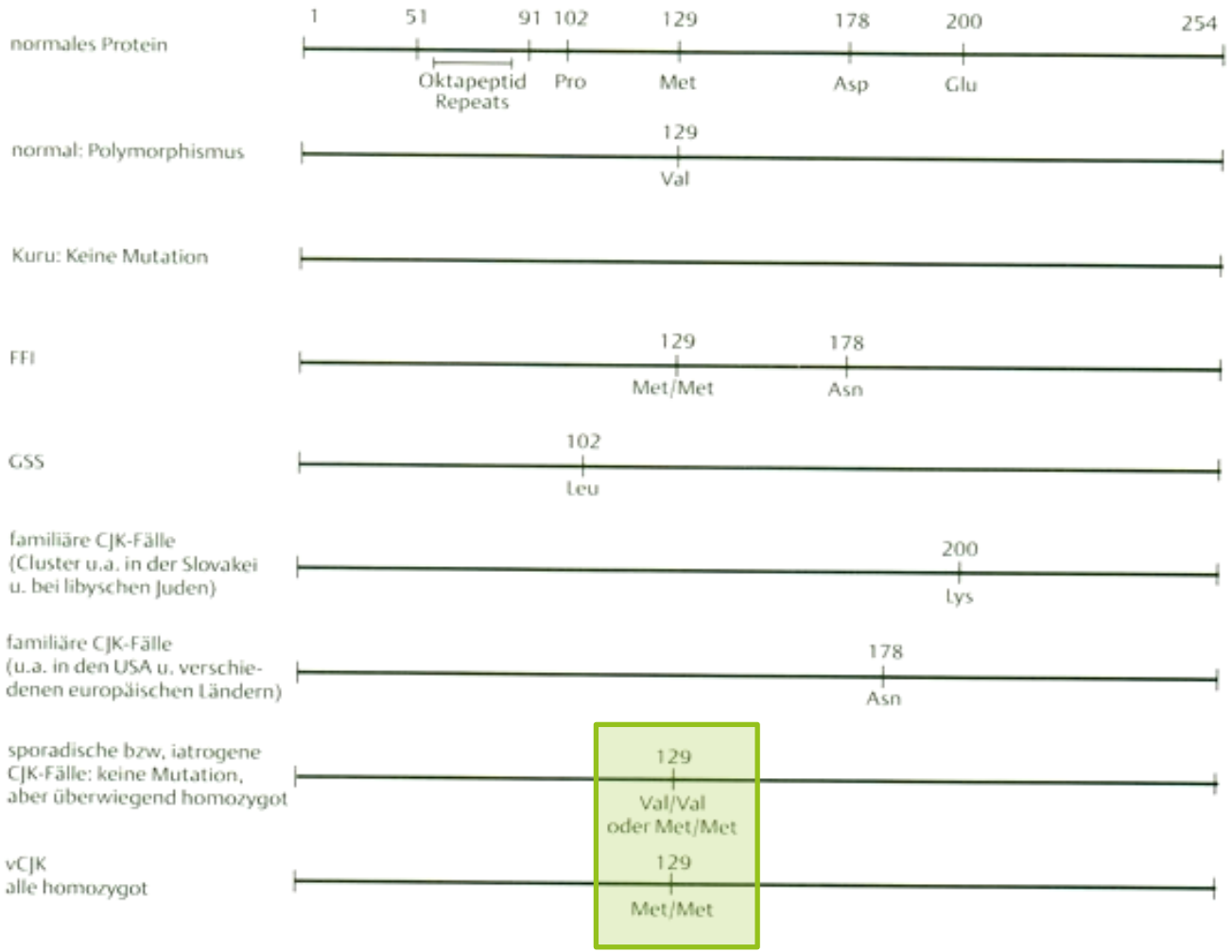
- Transgene mice for bovine PrP-Gene develop TSE after inoculation of brain-extracts of diseased cows
- Transgene mice with human PrP-Gene develop TSE after inoculation of brain-extracts of diseased cows
- Same incubation time, same neuropathology and same PrP-Isoforms after inoculation of brain-extracts of vCJD-Patients

  => Same strain?

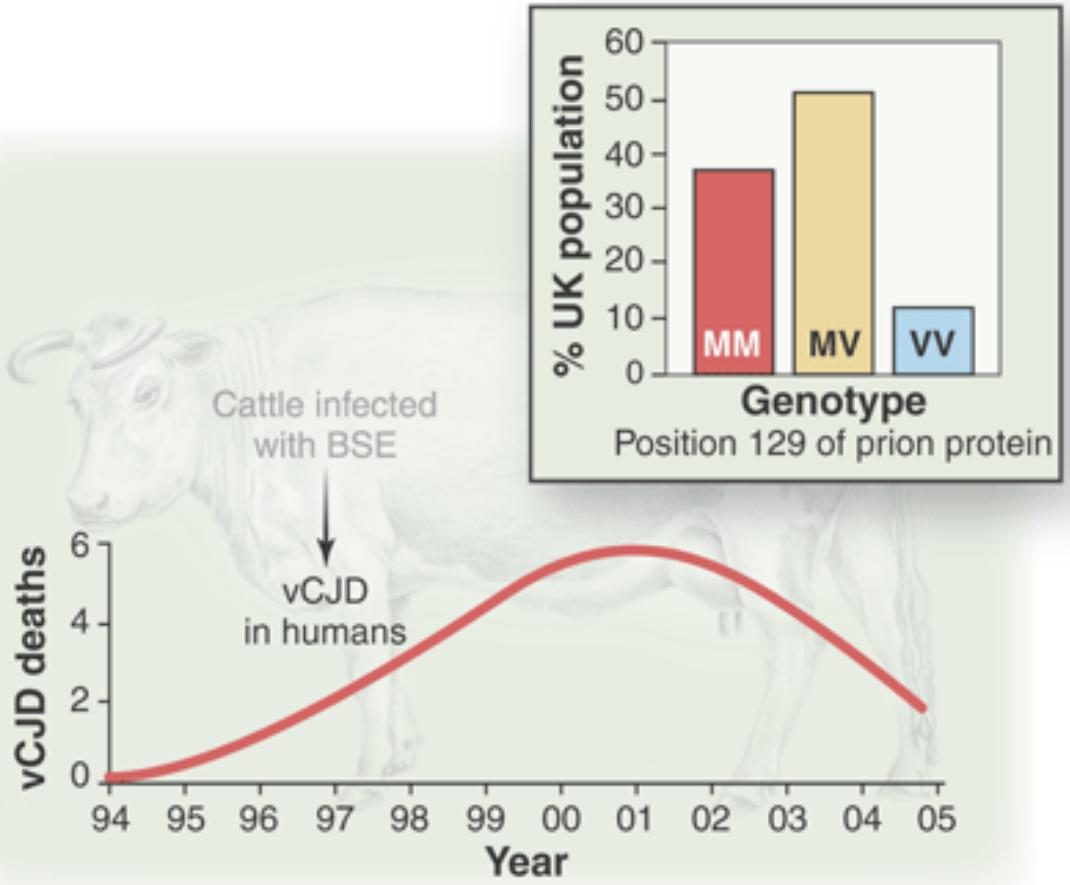
Conclusion (after 2006)

- Assumed 1.000.000 diseased cows, from which 460.000 entered the human food chain
- 164 vCJD in GB
- Single cases in other countries
- In Germany no case up to now
- Less than 200 BSE-cases reported
- 2006: 16 confirmed BSE cases in Germany
- 2007: 4
- 2008: 2

Mutations associated with human TSEs



Abhängigkeit vom Genotyp



All vCJD: 129MM

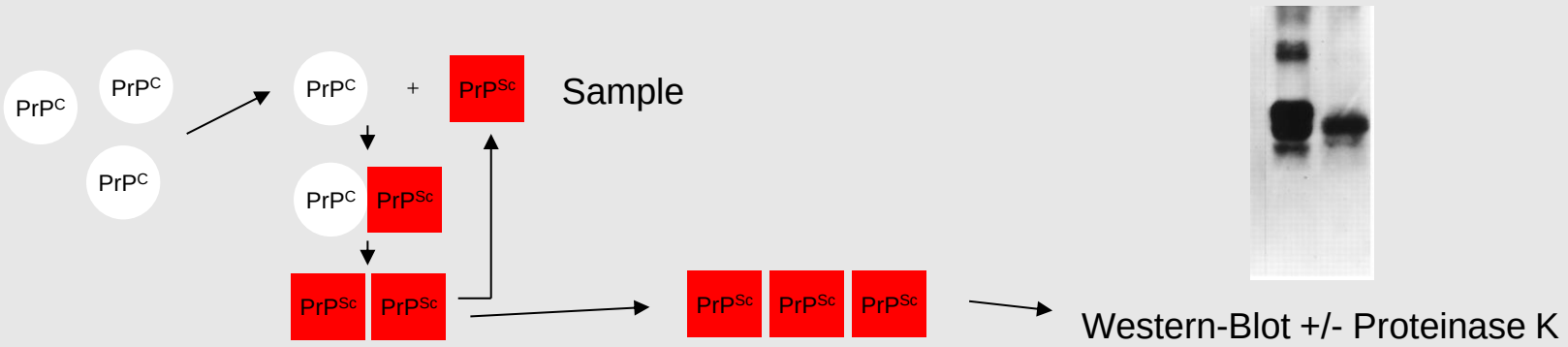
129MV:
3/12700 appendices:
PrP^{sc} positive =>
frequent latent form?

Blood transfusions of latter vCJD-Pat. in
129 MM => vCJD
129 MV => PrP^{Sc}
in lymph nodes

Prion Dormancy and Disease. Robin W. Carrell (2004) *Science* 306:1692-1693

Diagnostics of CJD

- Prion only in brain tissue but not in liquor detectable
- 14-3-3 marker protein in liquor by Western-Blot-Analyses reliable
- Neuropathologic diagnostics
  - Immuno histochemical staining with anti-PrP^{Sc}
  - Western-Blot-Analysis for Proteinase-K resistant PrP in brain extracts
- And: Protein Misfolding Cyclic Amplification (PMCA)



Open questions

- Are we still at the beginning of a vCJD outbreak?
  - Incubation period?
  - Prognoses for number of vCJD based on current data: < 403
- How is BSE transfered to humans?
  - M-cells – Lymphocytes – brain?
- Can vCJD be transmitted by blood, neurosurgery, transplantations?
  - Recommendations for sterilization of instrumentsand medical care of CJD-Patients
  - Exclusion of donor blood from people who lived in the beginning 90s for a longer time in GB
  - Infektivity before symptoms arise?

Präventionsmöglichkeiten und experimentelle Therapieansätze

- Species-appropriate feeding
- Prion-resistant cows (Knock-out; dominant-negative mutants)
- For CJD:
  - Gene therapy with dominant-negative mutants of PrP
  - Anti-Prion-antibodies

Security of blood products in Germany

- Actions taken:
  - 1994
    - Ban on imports of blood products for which production plasma from GB was used
  - 1998
    - “Transfusionsgesetz”
      - Declaration of origin (country)
      - Since 2003: Control of production conditions to removal of prions present in plasma
  - 2000
    - Leukocyte depletion
    - Exclusion of blood donors, which lived in GB between 1980 and 1996 or underwent surgery there
- Result:
  - Very low transmission risk of vCJK by blood products in Germany

Kursunterlagen

Moodle:

Kurs: Focal Point Molecular Medicine (SoSe25)

SpLect: Virology for Natural Scientists

Thank you...