2025 CJD FOUNDATION FAMILY CONFERENCE

JULY 11-13, 2025
CHICAGO, IL

Keynote address in honor of Pierluigi Gambetti, MD

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OXFORD

AUTOBIOGRAPHY

Autobiography Series: A Life of Anecdotes

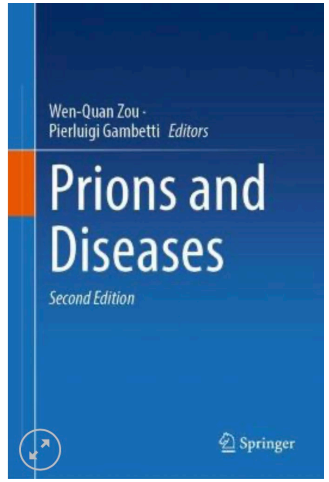
Pierluigi Gambetti, MD

Editors' Introduction

The following reminiscence by Pierluigi Gambetti is the sixteenth autobiography in a series published in the *Journal of Neuropathology and Experimental Neurology*. These have been solicited from senior members of the neuropathology community who have been noted leaders and contributors to neuroscience and to the American Association of Neuropathologists (AANP) and have a historical perspective of the importance of neuropathology in diagnosis, education, and research. We hope that this series will entertain, enlighten, and present members of the AANP with a better sense of the leg-

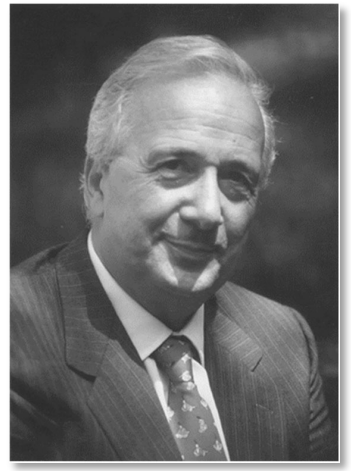
give it to you to translate you'll mess it up. Even worse. . . you will end up hating it." So, he gave us his great translations and challenged us to improve on them.

Upon entering Bologna University Medical School, my parents rented a room in the city so that I did not have to commute by train daily. I became part of a co-ed group of *provincial expats* and time flew. We all worked hard but we often had meals together at student restaurants with endless discussions about politics, movies that often we watched together, and other nonconsequential subjects. I did well in medical school and I graduated *magna cum laude*. However, my father



Pierluigi (right) with his older brother,
Imola, Italy, c. 1942

- Bologna University Medical School - *magna cum laude*
- Department of Neurology and Psychiatry, University of Bologna, Professor Elio Lugaresi
- February 1966 – McLean Hospital, Harvard. Met and married Dr. Lucila Autilio



Professor Elio Lugaresi

- June 1966 - joined the lab of Nick Gonatas, University of Pennsylvania to study EM
- Case Western Reserve University - Director of Neuropathology
- 1986 - Fatal familial insomnia and dysautonomia with selective degeneration of thalamic nuclei. *New England Journal of Medicine*
- 1992 - Fatal familial insomnia is a prion disease with a mutation at codon 178 of the prion protein gene. *New England Journal of Medicine*

- 1992 - Fatal familial insomnia and familial Creutzfeldt Jacob disease: disease phenotype determined by a DNA polymorphism. *Science*

Fatal Familial Insomnia and Familial Creutzfeldt-Jakob Disease: Disease Phenotype Determined by a DNA Polymorphism

Lev G. Goldfarb*, Robert B. Petersen*, Massimo Tabaton, Paul Brown, Andréa C. LeBlanc, Pasquale Montagna, Pietro Cortelli, Jean Julien, Claude Vital, William W. Pendelbury, Matti Haltia, Peter R. Wills, Jean J. Hauw, Paul E. McKeever, Lucia Monari, Bertold Schrank, Gary D. Swergold, Lucila Autilio-Gambetti, D. Carleton Gajdusek, Elio Lugaresi, Pierluigi Gambetti†

Fatal familial insomnia (FFI) and a subtype of familial Creutzfeldt-Jakob disease (CJD), two clinically and pathologically distinct diseases, are linked to the same mutation at codon 178 (Asn^{178}) of the prion protein gene. The possibility that a second genetic component modified the phenotypic expression of the Asn^{178} mutation was investigated. FFI and the familial CJD subtype segregated with different genotypes determined by the Asn^{178} mutation and the methionine-valine polymorphism at codon 129. The Met^{129}, Asn^{178} allele segregated with FFI in all 15 affected members of five kindreds whereas the Val^{129}, Asn^{178} allele segregated with the familial CJD subtype in all 15 affected members of six kindreds. Thus, two distinct disease phenotypes linked to a single pathogenic mutation can be determined by a common polymorphism.

In several Mendelian disorders, distinct phenotypes are linked to different point mutations in a single gene. Two forms of β-amyloid–related diseases, the hereditary cerebral hemorrhage with amyloidosis–Dutch type (HCHWA-D) and subtypes of familial Alzheimer's disease, are associated with different mutations in the amyloid precursor protein (APP) gene (*1*). Double point mutations in a single allele have been described in several conditions including sickle cell anemia, xeroderma pigmentosum, and GMI gangliosidosis (*2*). In these disorders, the second mutation triggers the disease or modifies its severity (*2*).

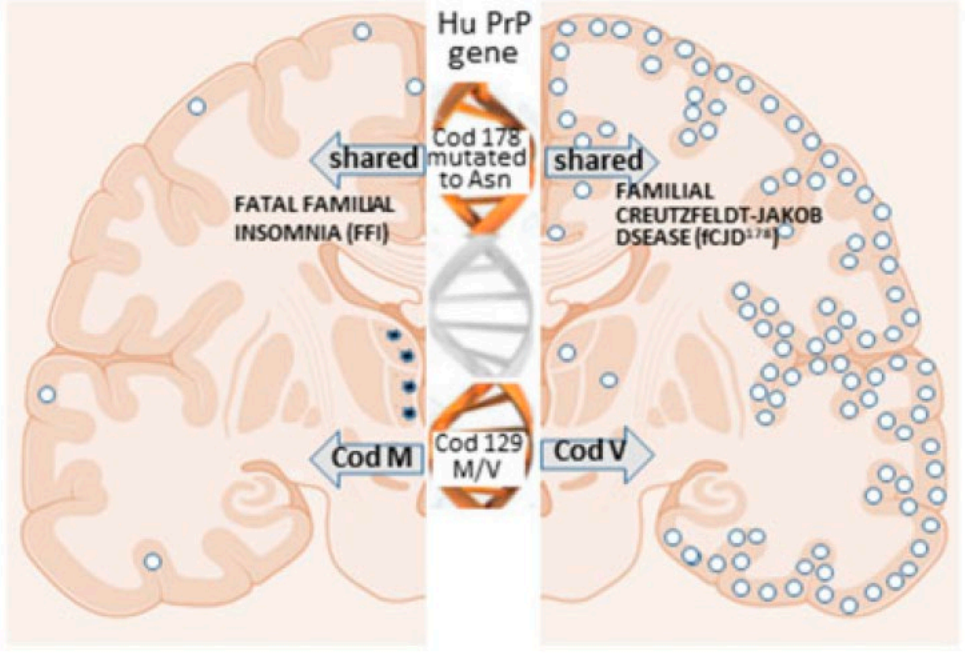
A group of inherited and sporadic disorders known as spongiform transmissible encephalopathies, or infectious amyloidoses, or prion diseases, are characterized by the presence of an abnormal isoform of the prion protein (PrP) that is resistant to proteases (*3*). PrP is encoded by a gene (*PRNP*) located on the short arm of human chromosome 20 (*3*). On the basis of clinical and pathological characteristics, three inherited forms of spongiform encephalopathies have been recognized: Gerstmann-Sträussler-Scheinker syndrome (GSS), characterized by chronic cerebellar ataxia and dementia in association with the presence of multicentric amyloid plaques (*4*); Creutzfeldt-Jakob disease (CJD), a subacute dementing illness with widespread spongiform degeneration (*4*); and the recently described fatal familial insomnia (FFI), a

L. G. Goldfarb, P. Brown, G. D. Swergold, D. C. Gajdusek, Laboratory of Central Nervous System Studies, National Institute of Neurological Diseases and Stroke, and Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.

The Gene With Two Faces

BY LORI OLIWENSTEIN

A rare genetic mutation has two methods of killing: it deprives you of sleep, permanently, or it makes you demented. Both methods work.



How do prions encode heritable strain information in the absence of nucleic acids?

Evidence for the Conformation of the Pathologic Isoform of the Prion Protein Enciphering and Propagating Prion Diversity

Glenn C. Telling, Piero Parchi, Stephen J. DeArmond, Pietro Cortelli, Pasquale Montagna, Ruth Gabizon, James Mastrianni, Elio Lugaresi, Pierluigi Gambetti, Stanley B. Prusiner*

The fundamental event in prion diseases seems to be a conformational change in cellular prion protein (PrP^C) whereby it is converted into the pathologic isoform PrP^{Sc}. In fatal familial insomnia (FFI), the protease-resistant fragment of PrP^{Sc} after deglycosylation has a size of 19 kilodaltons, whereas that from other inherited and sporadic prion diseases is 21 kilodaltons. Extracts from the brains of FFI patients transmitted disease to transgenic mice expressing a chimeric human-mouse PrP gene about 200 days after inoculation and induced formation of the 19-kilodalton PrP^{Sc} fragment, whereas extracts from the brains of familial and sporadic Creutzfeldt-Jakob disease patients produced the 21-kilodalton PrP^{Sc} fragment in these mice. The results presented indicate that the conformation of PrP^{Sc} functions as a template in directing the formation of nascent PrP^{Sc} and suggest a mechanism to explain strains of prions where diversity is encrypted in the conformation of PrP^{Sc}.

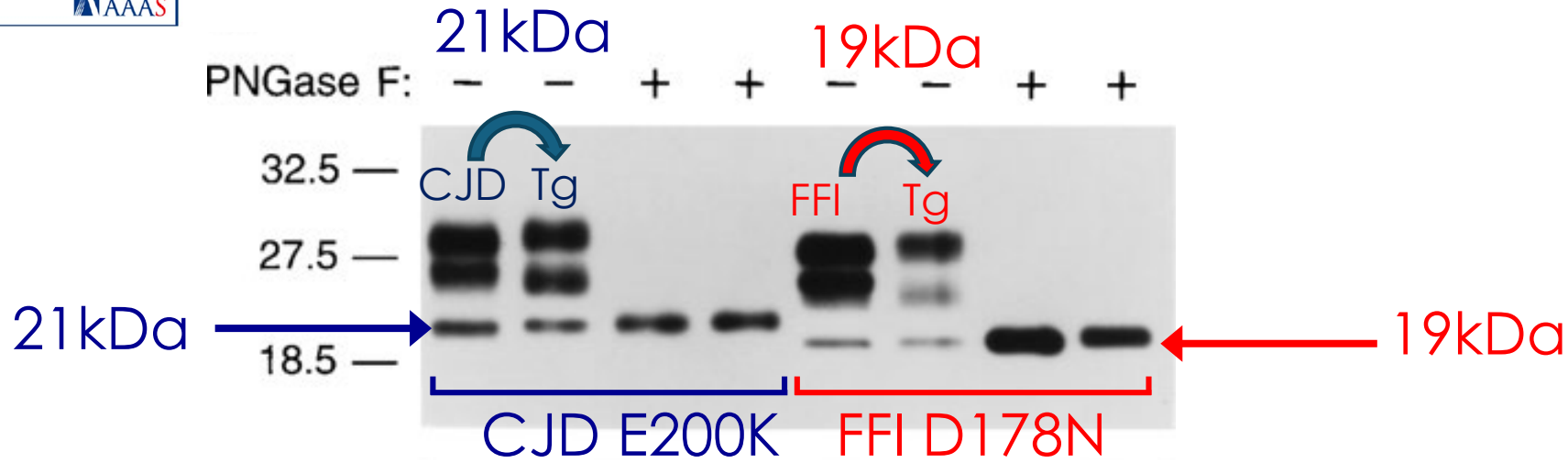
For many years the prion diseases, also called transmissible spongiform encephalopathies, were thought to be caused by slow-acting viruses (*1*), but it is now clear that prions are not viruses and that they are devoid of nucleic acid (*2*, *3*). Prions seem to be composed only of PrP^{Sc} molecules, which are abnormal conformers of a normal, host-encoded protein designated PrP^C (*3*, *4*). PrP^C has a high α-helical content and is virtually devoid of β-sheets, whereas PrP^{Sc} has a high β-sheet content (*4*, *5*); thus, the conversion of PrP^C into PrP^{Sc} involves a profound conformational change. Formation of PrP^{Sc} is a posttranslational process that does not appear to involve a covalent modification of the protein (*6*).

The prion diseases are unique in that they may present as inherited and infectious disorders (*3*, *7*). More than 20 different mutations of the human (Hu) PrP gene segregate with dominantly inherited disease; five of these have been genetically linked to familial Creutzfeldt-Jakob disease (fCJD), Gerstmann-Sträussler-Scheinker disease, and fatal familial insomnia (FFI) (*8*). The most common prion diseases of animals are scrapie of sheep and bovine spongiform encephalopathy; the latter may have been transmitted to people through foods (*9*).

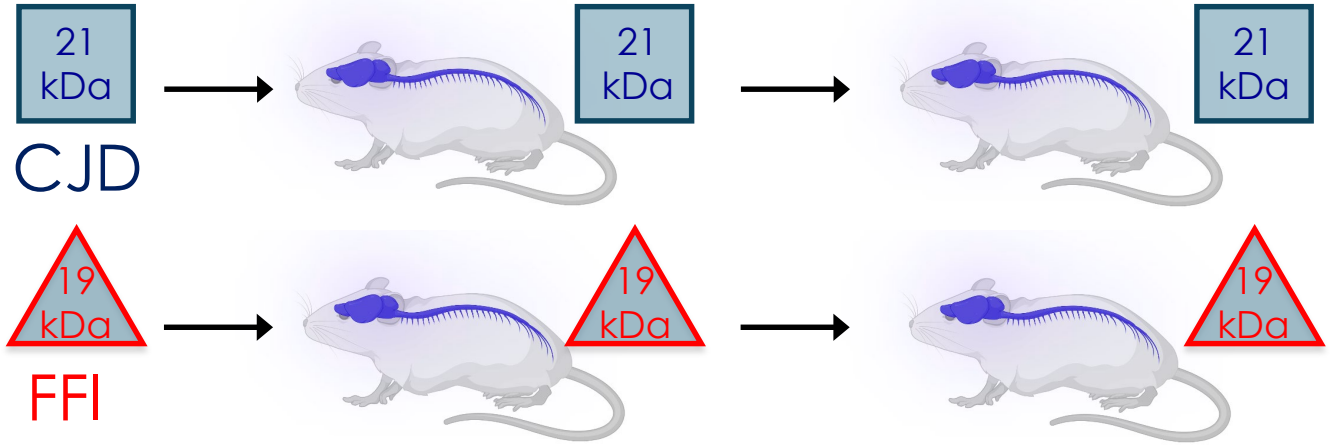
To extend studies on the transmission of

G. C. Telling and J. Mastrianni, Department of Neurology, University of California, San Francisco, CA 94143, USA. P. Parchi and P. Gambetti, Division of Neuropathology, Department of Pathology, Case Western Reserve University, Cleveland, OH 44106, USA. S. J. DeArmond, Departments of Neurology and Pathology, University of California, San Francisco, CA 94143, USA. P. Cortelli, P. Montagna, E. Lugaresi, Department of Neurology, University of Bologna, Bologna 40123, Italy. R. Gabizon, Department of Neurology, Hadassah Medical Center, Hebrew University, Jerusalem 91120, Israel. S. B. Prusiner, Departments of Neurology and Biochemistry and Biophysics, University of California, San Francisco, CA 94143, USA.

*To whom correspondence should be addressed at the Department of Neurology, HSE-781, University of California, San Francisco, CA 94143-0518, USA.



Strain information is enciphered within distinct prion protein conformations which are faithfully templated during prion replication

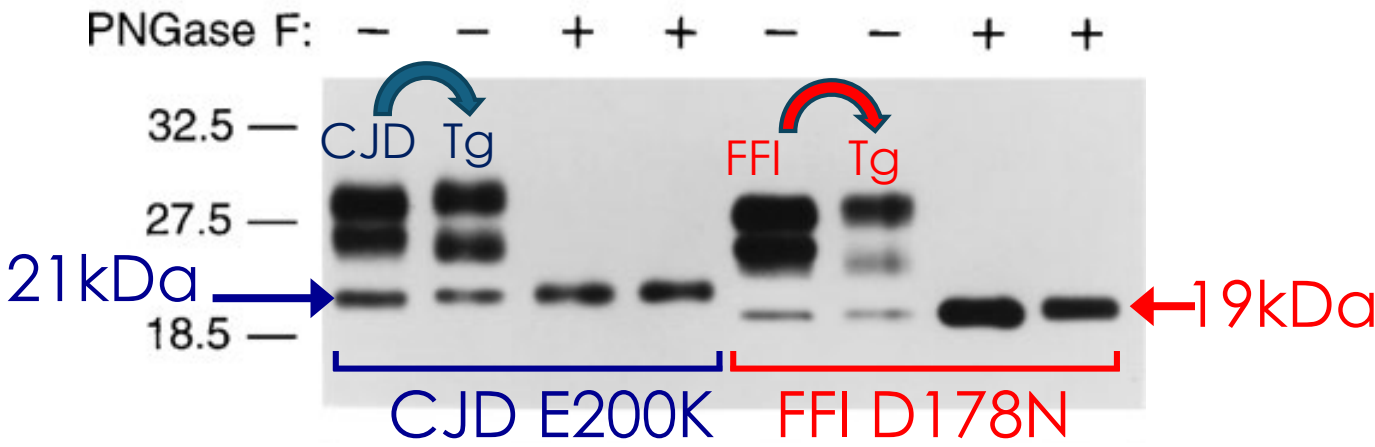


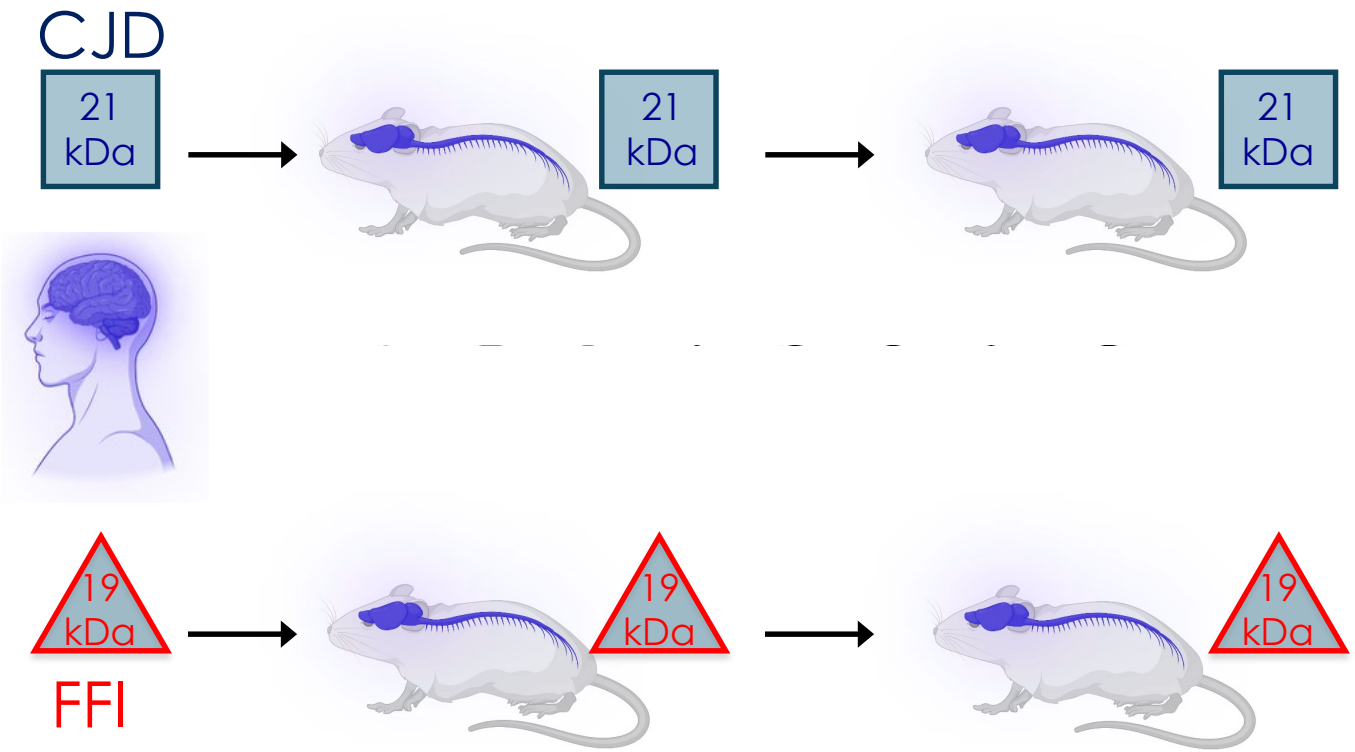
'We decided to collaborate on (transmission of FFI) with Stanley Prusiner, who seemed to have the best transgenic (Tg) mice expressing human PrP.

Piero Parchi, a talented and productive postdoctoral fellow from Bologna, and I could not believe our eyes: Tg mice inoculated with fCJD200 or FFI brain homogenates displayed resPrP^{D} type 1 or 2 mimicking those of the fCJD200 and FFI patients. This finding showed that human resPrP^{D} types were reproducible despite the different (mouse vs human) brain microenvironments.

Stan quickly observed that type 1 and 2 reproductions also occurred in the absence of the pathogenic mutation of FFI and fCJD200, which the Tg mice did not carry. Therefore, the inoculated human resPrP^{D} types 1 and 2 carried all the information needed to reproduce themselves in the Tg mice with no apparent nucleic acid intervention.

In my opinion, the results of this study published in 1996 significantly strengthened the 'protein-only or prion' hypothesis for which Stan deservedly received the 1997 Nobel Prize.'





- 1999 - Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. *Annals of Neurology*

- 2008 and 2010 - A novel human disease with abnormal prion protein sensitive to protease. Variably protease-sensitive prionopathy: a new sporadic disease of the prion protein. *Annals of Neurology*

- 1997 - National Prion Disease Pathology Surveillance Center established at CWRU under the aegis of the Centers for Disease Control and Prevention. Nearly 19-year directorship.

https://doi.org/10.1093/brain/awad399 BRAIN 2024: 147; 1539–1552 | 1539

BRAIN
ORIGINAL ARTICLE

Efficient transmission of human prion diseases to a glycan-free prion protein-expressing host

Laura Cracco,[1] Ignazio Cali,[2,3] Mark L. Cohen,[2] Rabail Aslam,[2] Silvio Notari,[2] Qingzhong Kong,[2,3,4] Kathy L. Newell,[1] Bernardino Ghetti,[1] Brian S. Appleby[2,3,4,5] and Pierluigi Gambetti[2]

It is increasingly evident that the association of glycans with the prion protein (PrP), a major post-translational modification, significantly impacts the pathogenesis of prion diseases. A recent bioassay study has provided evidence that the presence of PrP glycans decreases spongiform degeneration and disease-related PrP (PrP^D) deposition in a murine model. We challenged (*PRNP*$^{N181Q/197Q}$) transgenic (Tg) mice expressing glycan-free human PrP (TgGlyc–), with isolates from sporadic Creutzfeldt–Jakob disease subtype MM2 (sCJDMM2), sporadic fatal insomnia and familial fatal insomnia, three human prion diseases that are distinct but share histotypic and PrP^D features. TgGlyc– mice accurately replicated the basic histotypic features associated with the three diseases but the transmission was characterized by high attack rates, shortened incubation periods and a greatly increased severity of the histopathology, including the presence of up to 40 times higher quantities of PrP^D that formed prominent deposits. Although the engineered protease-resistant PrP^D shared at least some features of the secondary structure and the presence of the anchorless PrP^D variant with the wild-type PrP^D, it exhibited different density gradient profiles of the PrP^D aggregates and a higher stability index. The severity of the histopathological features including PrP deposition appeared to be related to the incubation period duration. These findings are clearly consistent with the protective role of the PrP glycans but also emphasize the complexity of the conformational changes that impact PrP^D following glycan knockout. Future studies will determine whether these features apply broadly to other human prion diseases or are PrP^D-type dependent.

CREUTZFELDT-JAKOB DISEASE FOUNDATION, INC.
Supporting Families Affected by Prion Disease

CJD Surveillance
National Prion Disease
Pathology Surveillance Center

Pierluigi and the CJD Foundation

'The CJD Foundation was moved to Akron (OH) and very successfully expanded by the foundation president, Ms. Florence Kranitz, creating the conditions for a very productive collaboration with the National Prion Disease Pathology Surveillance Center. One of Florence's most important initiatives was the annual CJD Foundation Family Conference. The three days spent with patients' families was a special time, moving and very enlightening. It gave me a glimpse of the other side of human prion diseases, and of the profound sorrow and pain they cause. I believe that this experience gave me a better sense of my work. I encourage physicians and biomedical researchers not in direct contact with patients and their families to seek out similar experiences.'

Neil Cashman Pierluigi Florence Kranitz

A valued colleague

Sandra Pritzkow | Claudio Soto | Ed Hoover | Jason Bartz | Pierluigi | Luci

Manhattan, New York, September 2017

A valued colleague

Candace Mathiason

Jason Bartz

Pierluigi

Claudio Soto

Santa Fe, New Mexico, October 2022

prion research center

CREUTZFELDT-JAKOB DISEASE FOUNDATION, INC.

Supporting Families Affected by Prion Disease

National Institutes of Health

Novel mouse models of prion diseases

Glenn Telling
Prion Research Center
Colorado State University

Creutzfeldt Jakob Disease Foundation Family Conference
Chicago, IL, 2025

Candace Mathiason - CSU

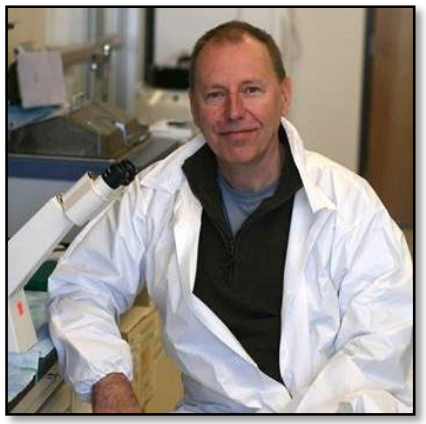
Jennifer Malmberg - USDA

Mark Zabel - CSU

Amanda Woerman - CSU

PRC

prion research center



Glenn Telling - CSU

Julie Moreno - CSU

Jason Bartz - Creighton

Eric Ross - CSU

Prions – Transmissible spongiform encephalopathies

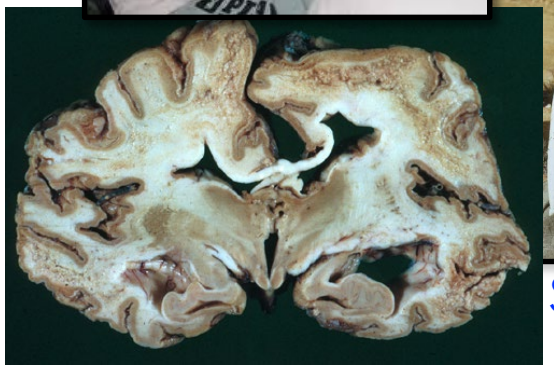
Scrapie

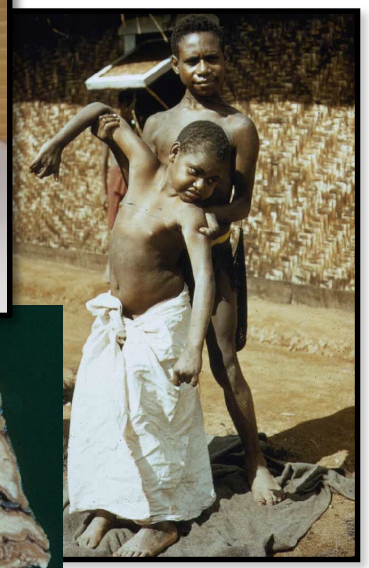
Bovine spongiform encephalopathy

Chronic wasting disease

Transmissible mink encephalopathy

Variant Creutzfeldt Jakob disease





Kuru

Sporadic Creutzfeldt Jakob disease

- Prion diseases are fatal, transmissible neurodegenerative disorders which frequently occur as epidemics
- Prions epitomize an unprecedented mechanism of protein-mediated information transfer
- The capacity for prions to transmit between species is unpredictable

Prions – Transmissible spongiform encephalopathies

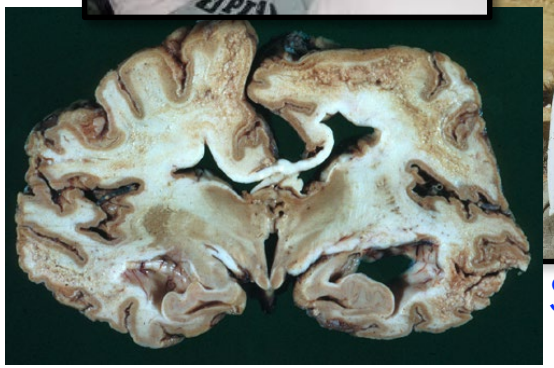
Scrapie

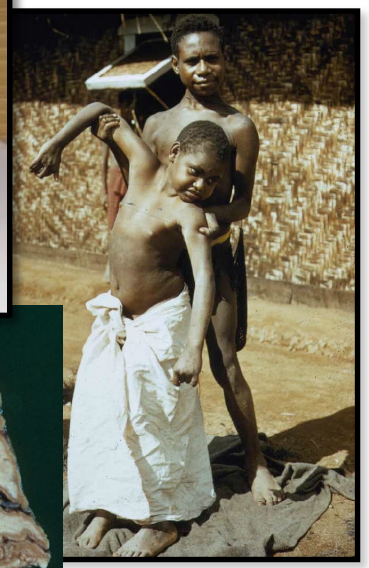
Bovine spongiform encephalopathy

Chronic wasting disease

Transmissible mink encephalopathy

Variant Creutzfeldt Jakob disease





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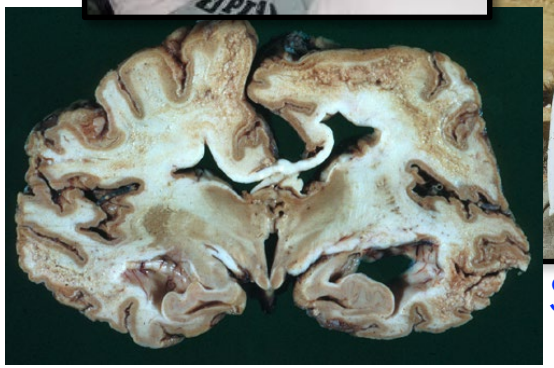

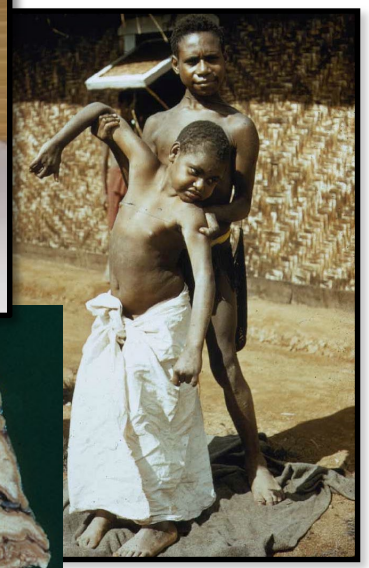

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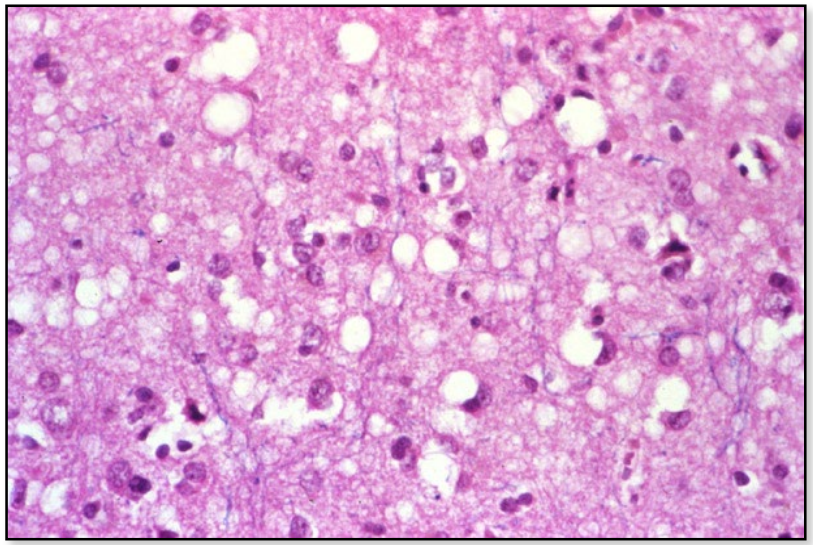
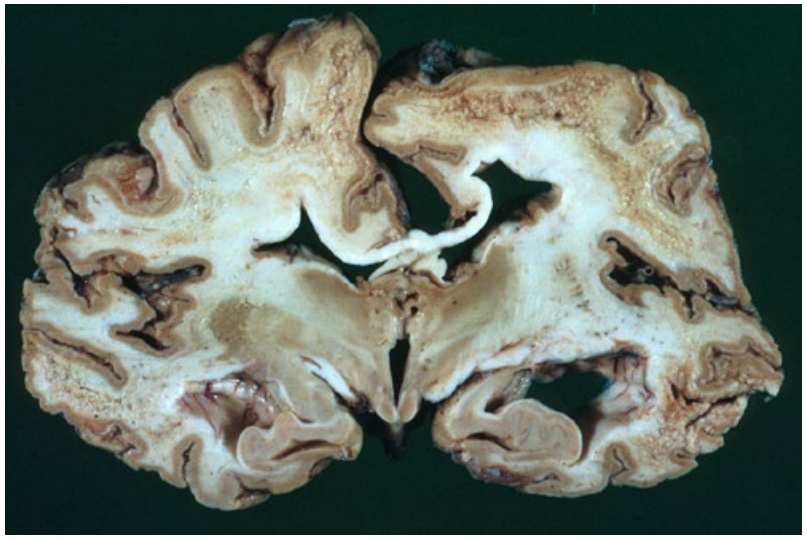
Prions – cause fatal, transmissible neurodegenerative diseases of the central nervous system

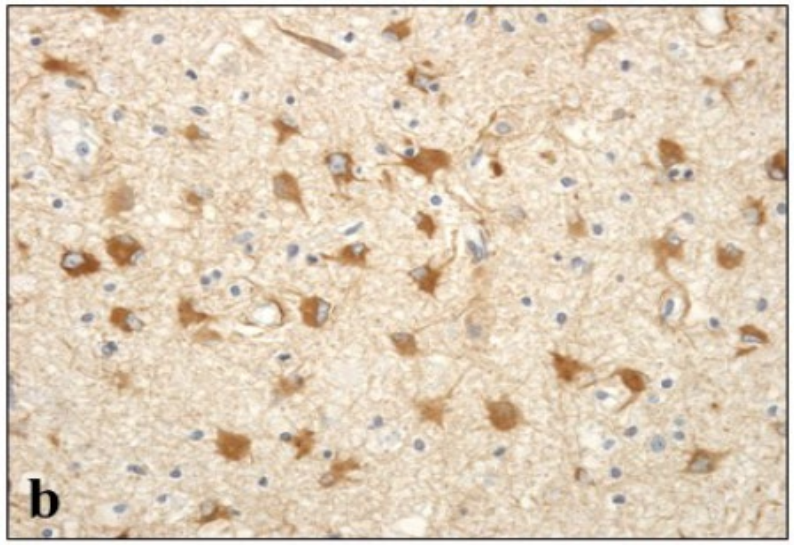
Spongiform degeneration

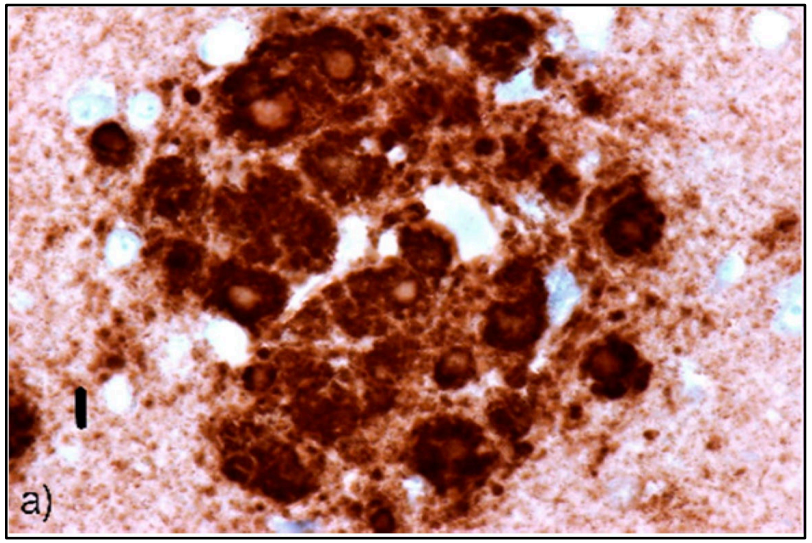




Massive neuronal loss

Reactive astrogliosis





Amyloid plaques

Transmissible features of TSEs were consistent with a viral etiology

Features suggesting a viral aetiology

- Transmissible with a rapidly progressive clinical phase
- Similar clinical presentations
- Agent is filterable and titratable
- Agent exhibits strain properties

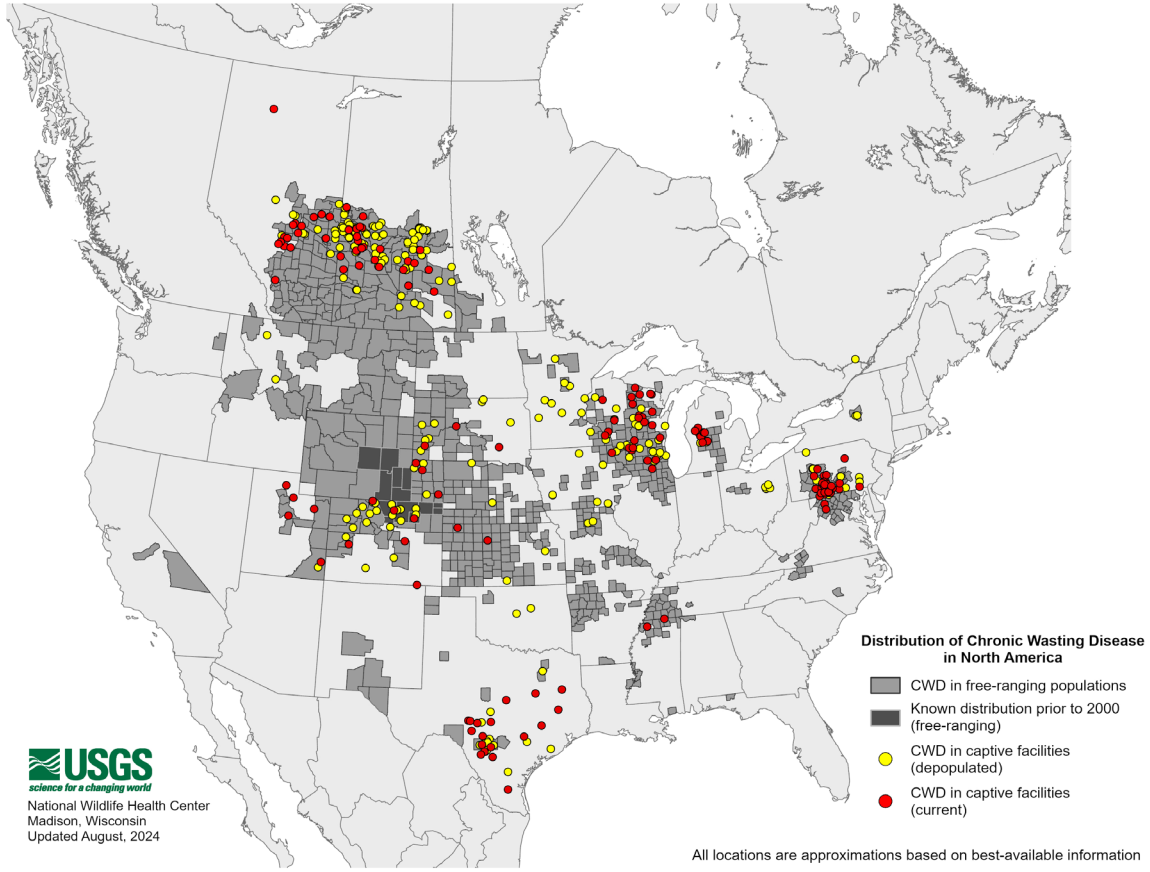
Experimental transmission of scrapie infection

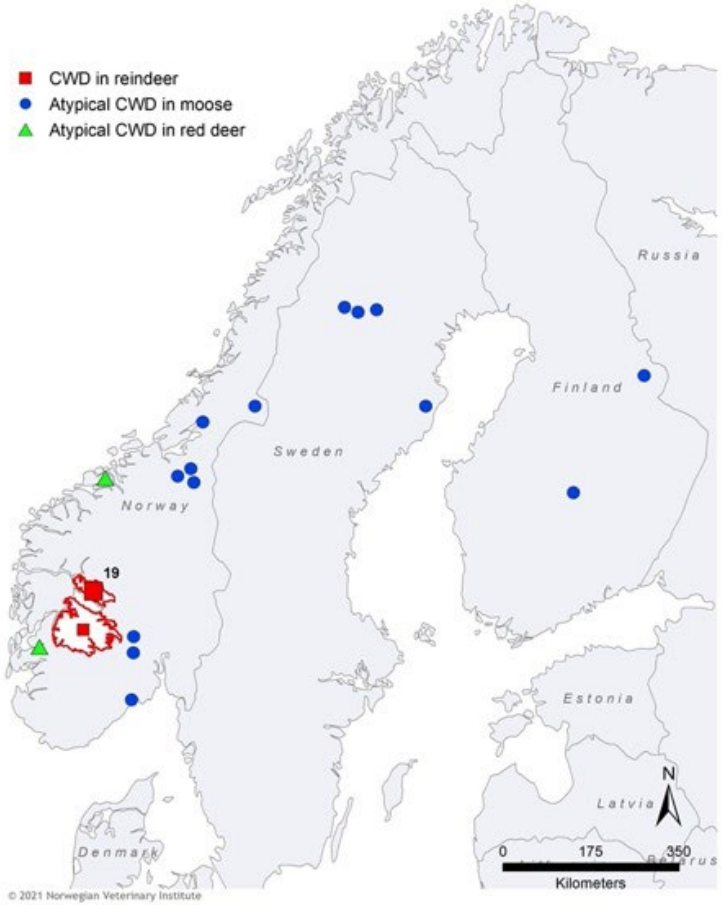
France 1936

Jean Cuille

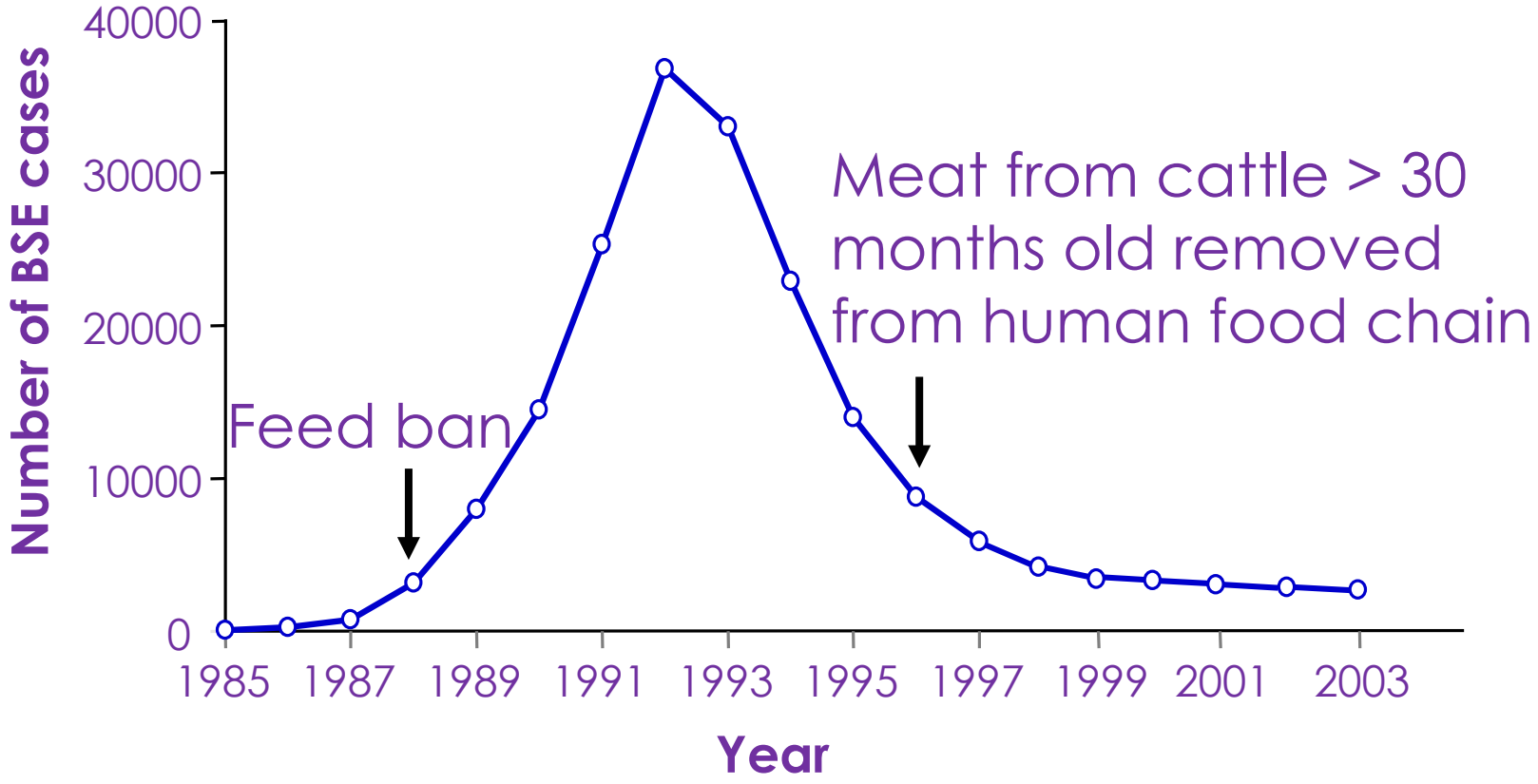
Paul-Louis Chelle

CWD - an emerging global prion disease



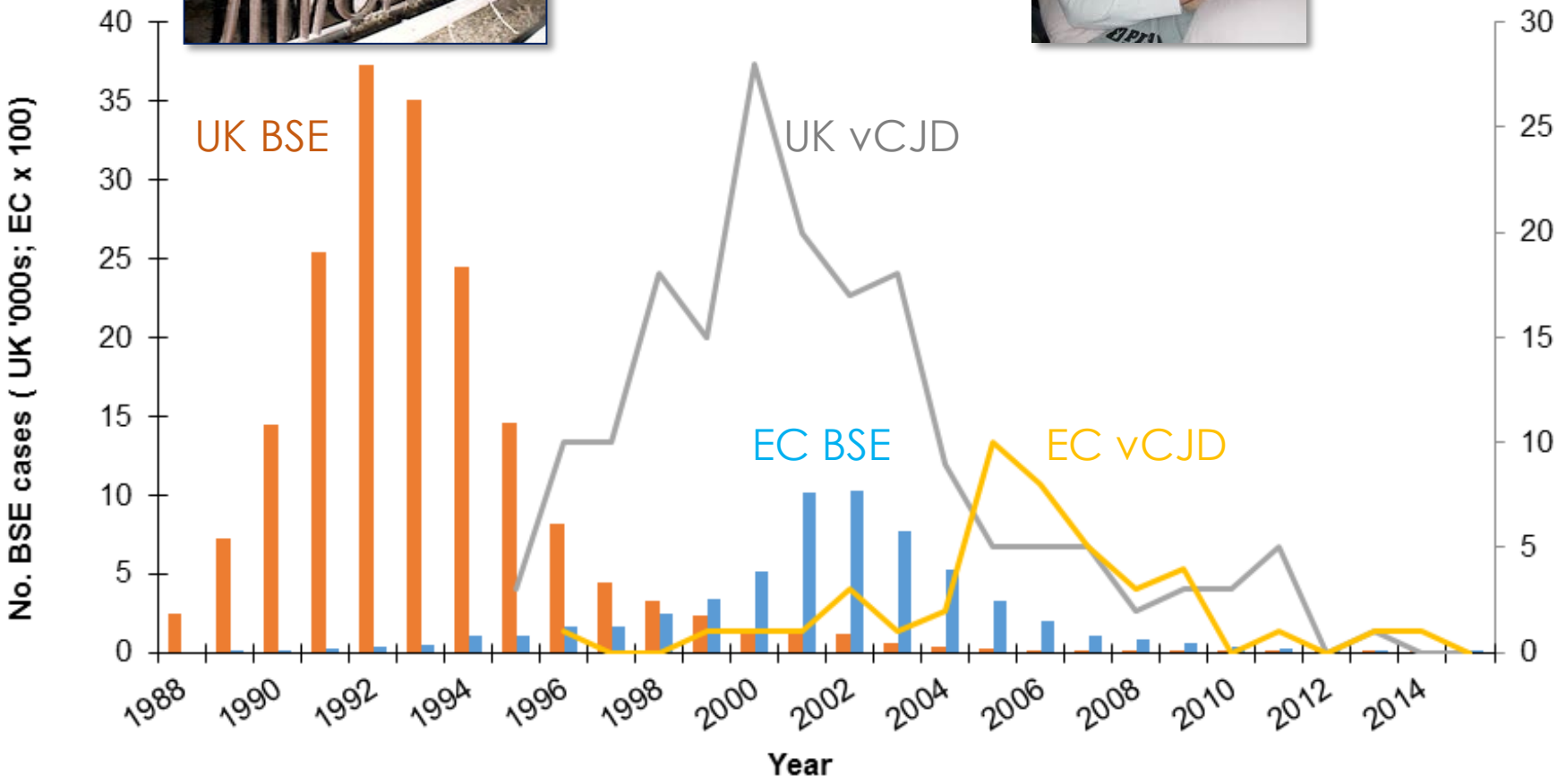


Foodborne transmission of bovine spongiform encephalopathy (BSE) – mad cow disease



- Jan 1993 - Epidemic peaked at ~ 1,000 new cases /week
- By Dec. 1, 2000 - 177,531 cattle diagnosed with BSE in Great Britain

Zoonotic transmission of BSE to humans – variant Creutzfeldt-Jakob disease (vCJD)



Other human prion diseases acquired by infection

Iatrogenic CJD

Accidental human prion transmission during medical procedures

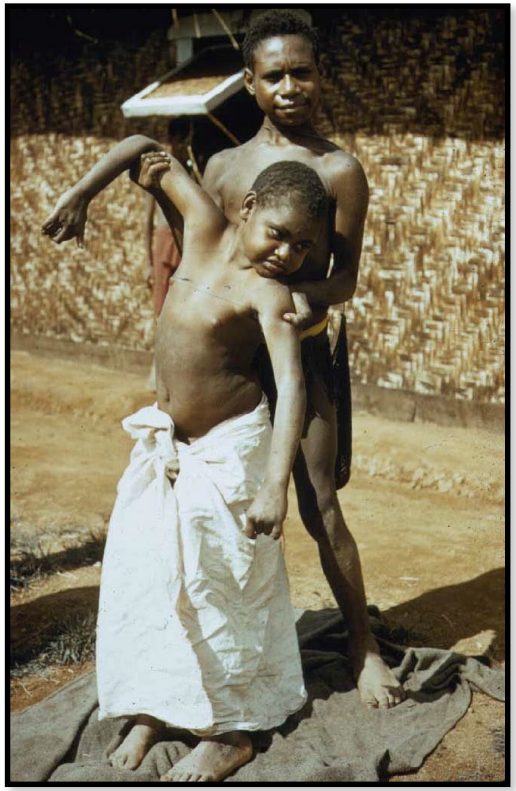
Aetiology:

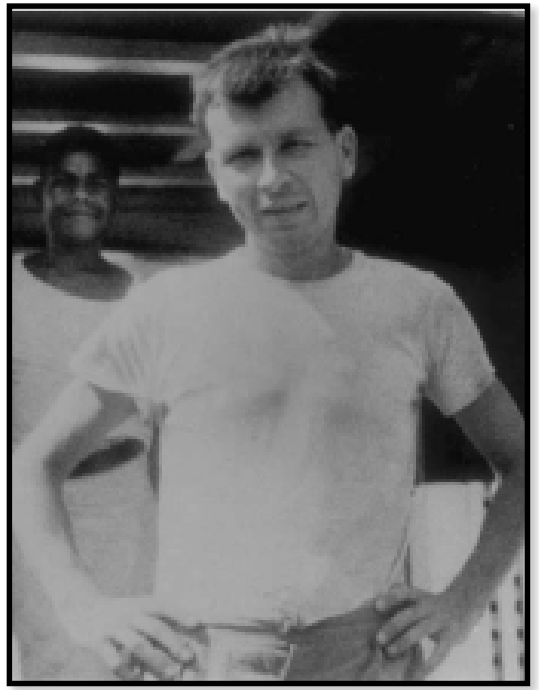
143 cases	- Human pituitary growth hormone
4 cases	- Human pituitary gonadotropin
169 cases	- Dura mater grafts
3 cases	- Cornea transplants
2 cases	- EEG electrodes
4 cases	- Neurosurgery

Other human prion diseases acquired by infection

Kuru

Epidemic among the Fore peoples of Papua New Guinea (>2500 cases)

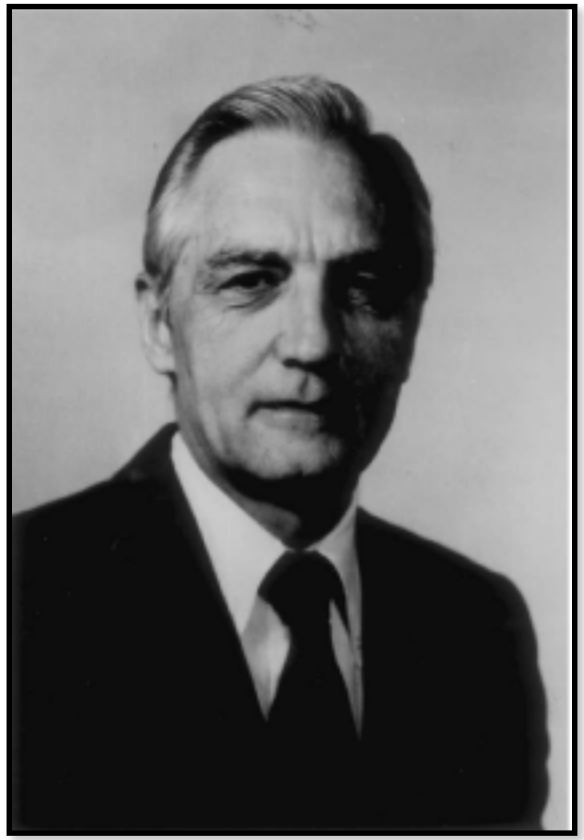




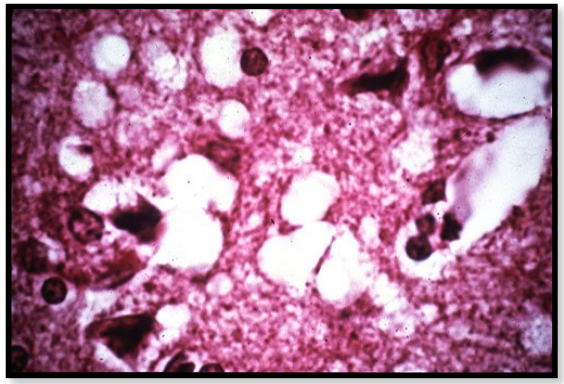
Carleton Gajdusek

Aetiology: Ritualistic cannibalism

Experimental transmission of human prion diseases to non-human primates



William Hadlow

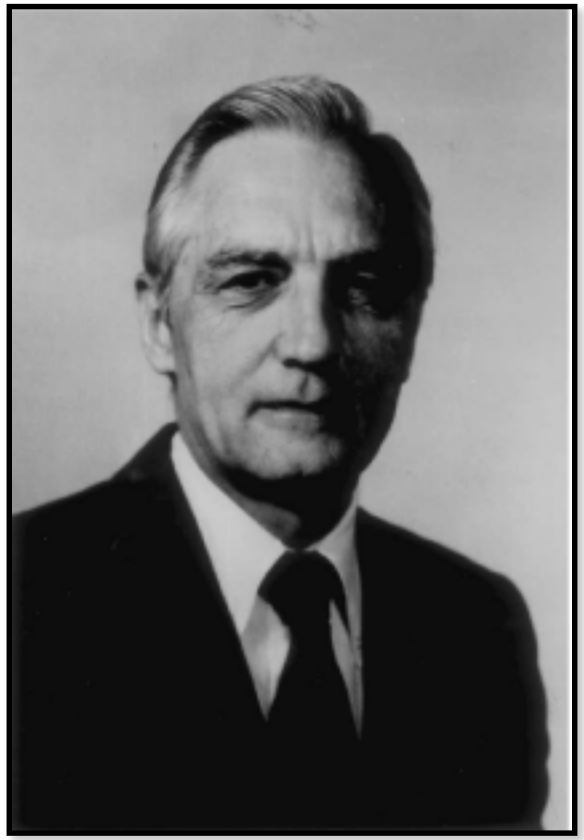


Neuropathological similarities of Kuru and scrapie

Clarence Gibbs

Experimental transmission of human prion diseases to non-human primates

United States 1959-1968



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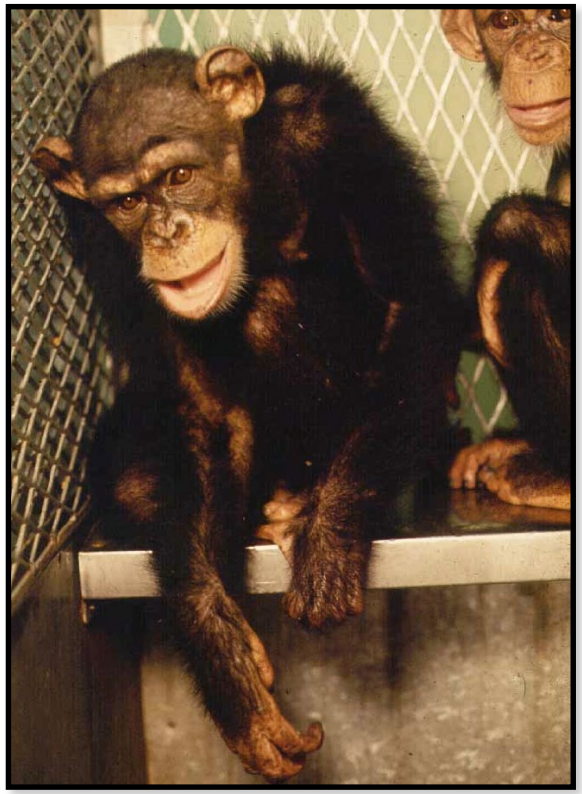
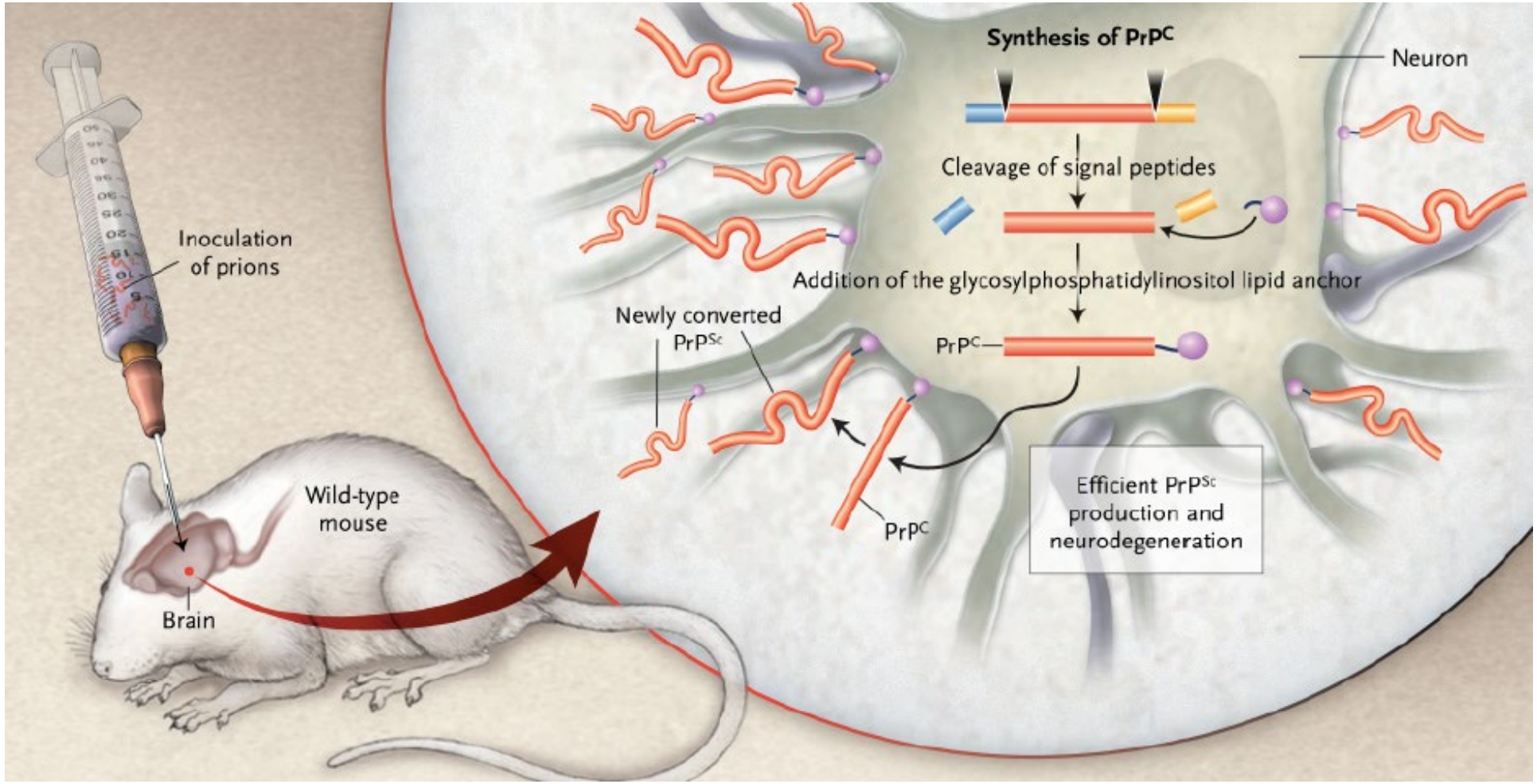


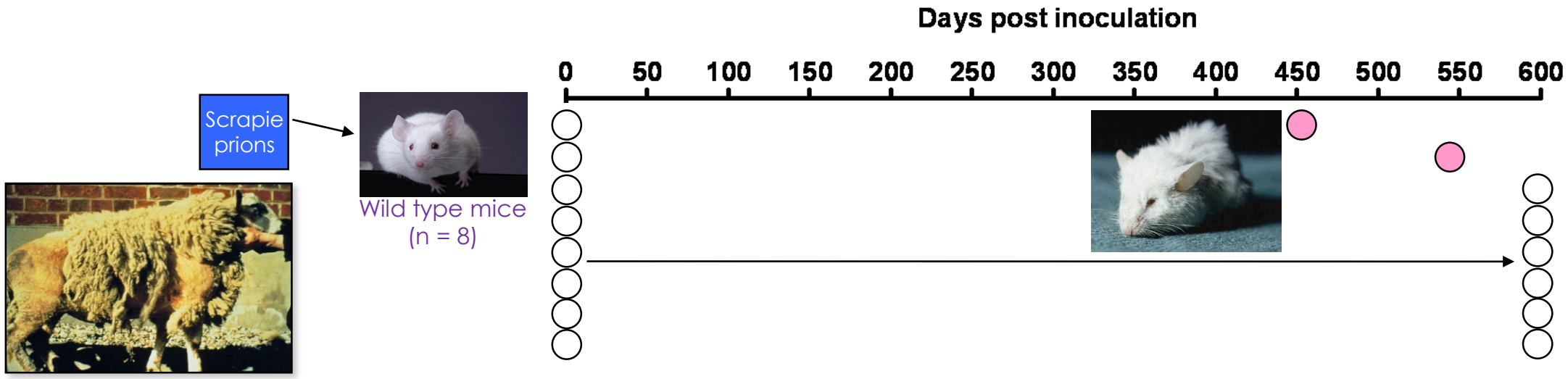
Clarence Gibbs

Experimental transmission of Prions to Mice



The species barrier in prion transmission

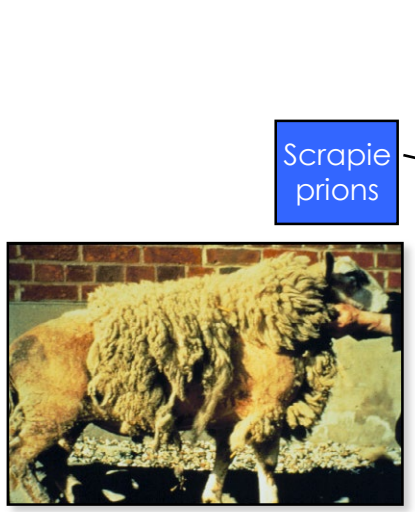
Although infection is transmitted readily to animals of the same species, interspecies transmission is generally more difficult. **Species Barrier**



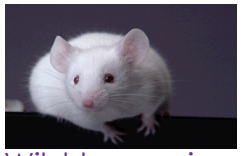
- Generally low attack rate
- Prolonged incubation times in diseased animals
- High variance of times to onset of disease

The species barrier in prion transmission

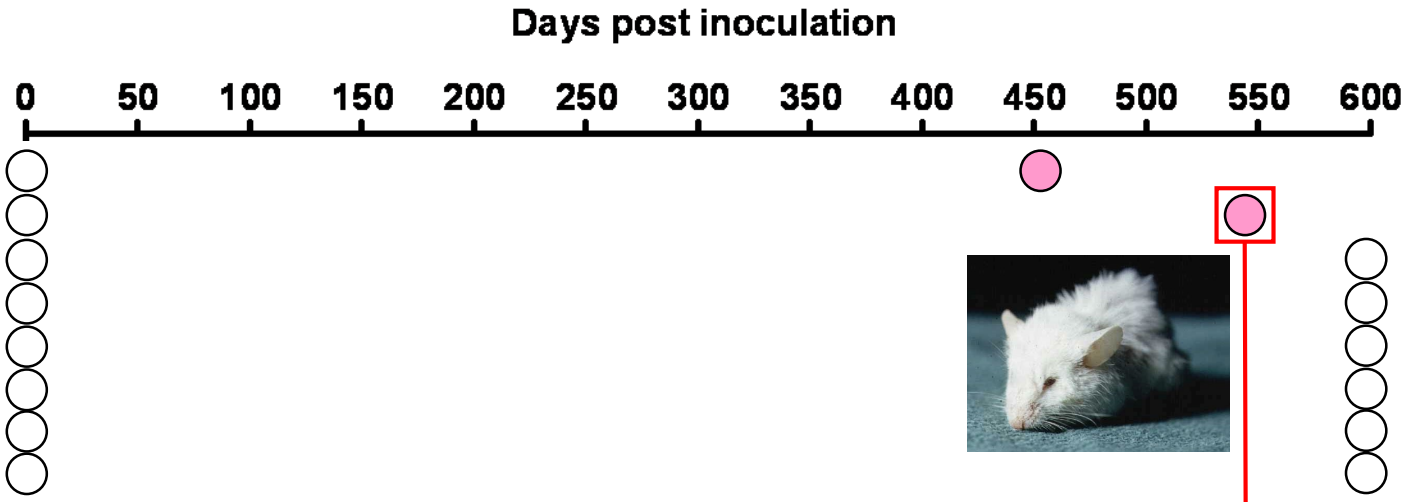
Species Barrier



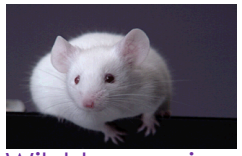
Scrapie prions



Wild type mice
(n = 8)



Mouse-adapted
Scrapie prions

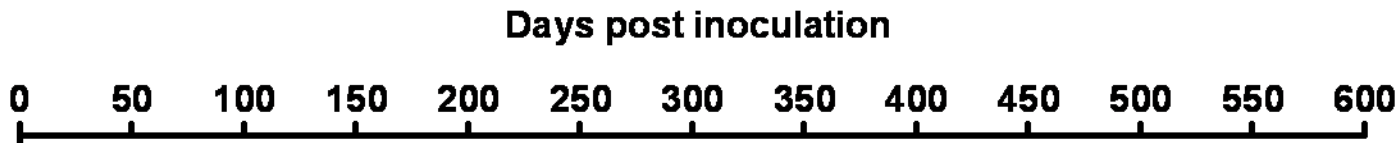


Wild type mice
(n = 8)

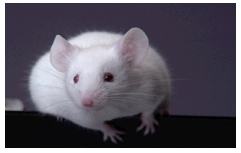
- 100 % attack rate
- Incubation time 170±3d (8/8)
- SEM < 2 % of mean

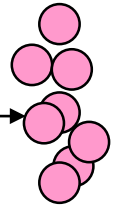
Mouse-adapted
Scrapie prions
NEW STRAIN

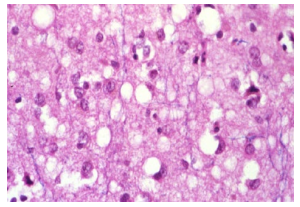
Strain properties were consistent with a viral etiology



Strain A



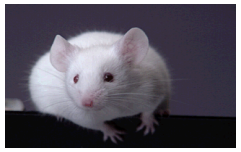


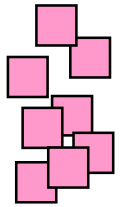


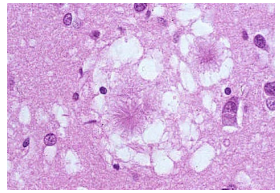
- Incubation time
- Neuropathology

140 ± 2d (8/8)
Type A

Strain B



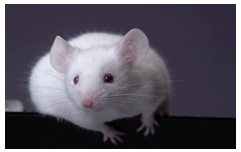


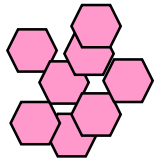


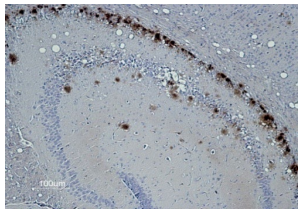
- Incubation time
- Neuropathology

250 ± 3d (8/8)
Type B

Strain C





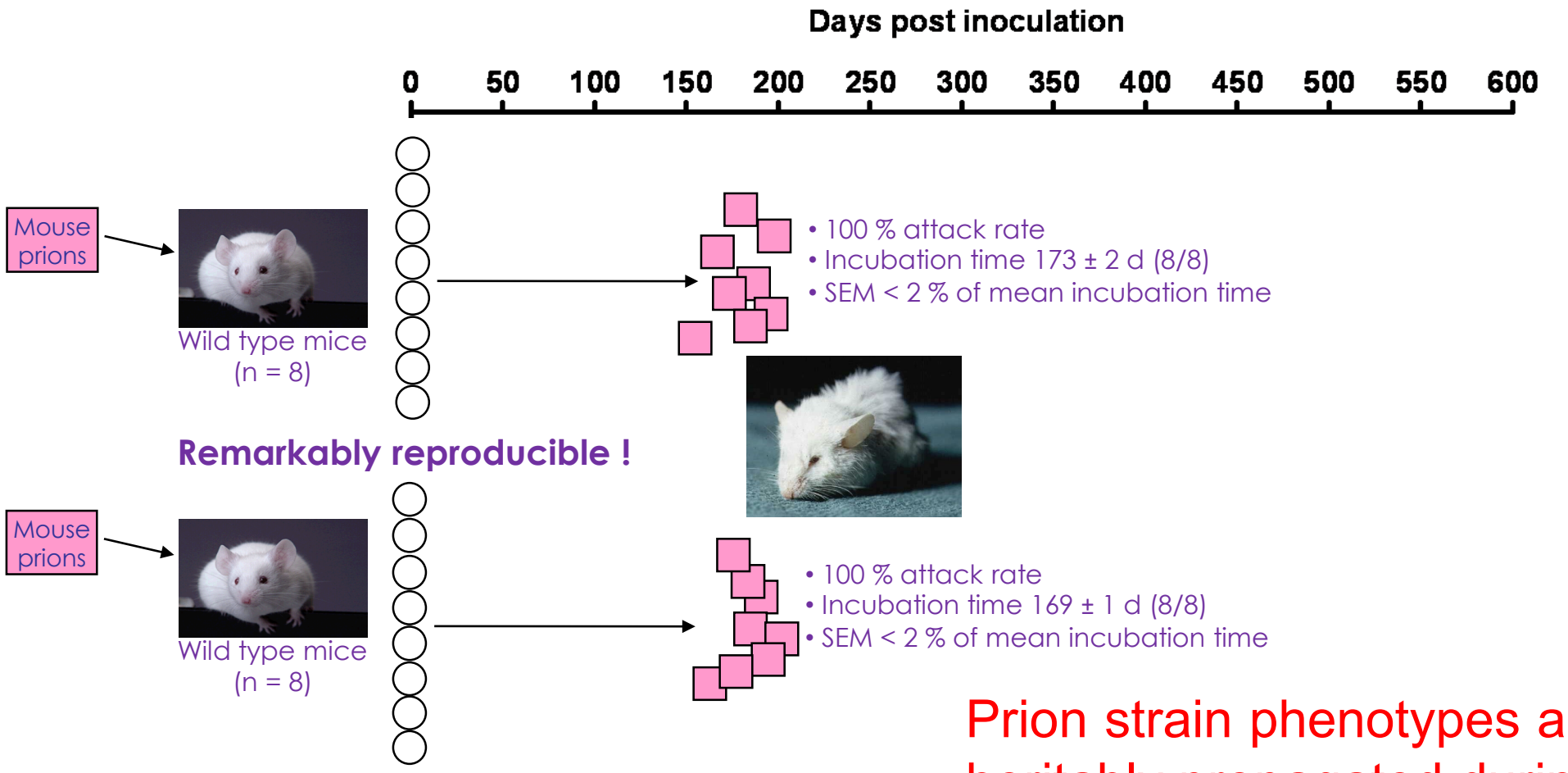


- Incubation time
- Neuropathology

410 ± 5d (8/8)
Type C

These prion strain phenotypes are heritably propagated during iterative transmissions.

Prion transmissibility was consistent with a viral etiology



Prion strain phenotypes are heritably propagated during iterative transmissions.

Unique characteristics of TSEs suggested a highly unusual infectious agent

Unusual features of the infectious agent that were inconsistent with viral infection

- Absence of immune response
- Agent extremely resistant to inactivation
- Extremely long incubation times
- Agent contains no detectable nucleic acid

Kuru incubation times exceeding five decades

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

Phil. Trans. R. Soc. B (2008) **363**, 3725–3739
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Published online

A clinical study of kuru patients with long incubation periods at the end of the epidemic in Papua New Guinea

John Collinge[1,*], Jerome Whitfield[1,2,3], Edward McKintosh[1], Adam Frosh[1], Simon Mead[1], Andrew F. Hill[1,†], Sebastian Brandner[1], Dafydd Thomas[1] and Michael P. Alpers[1,2,3]

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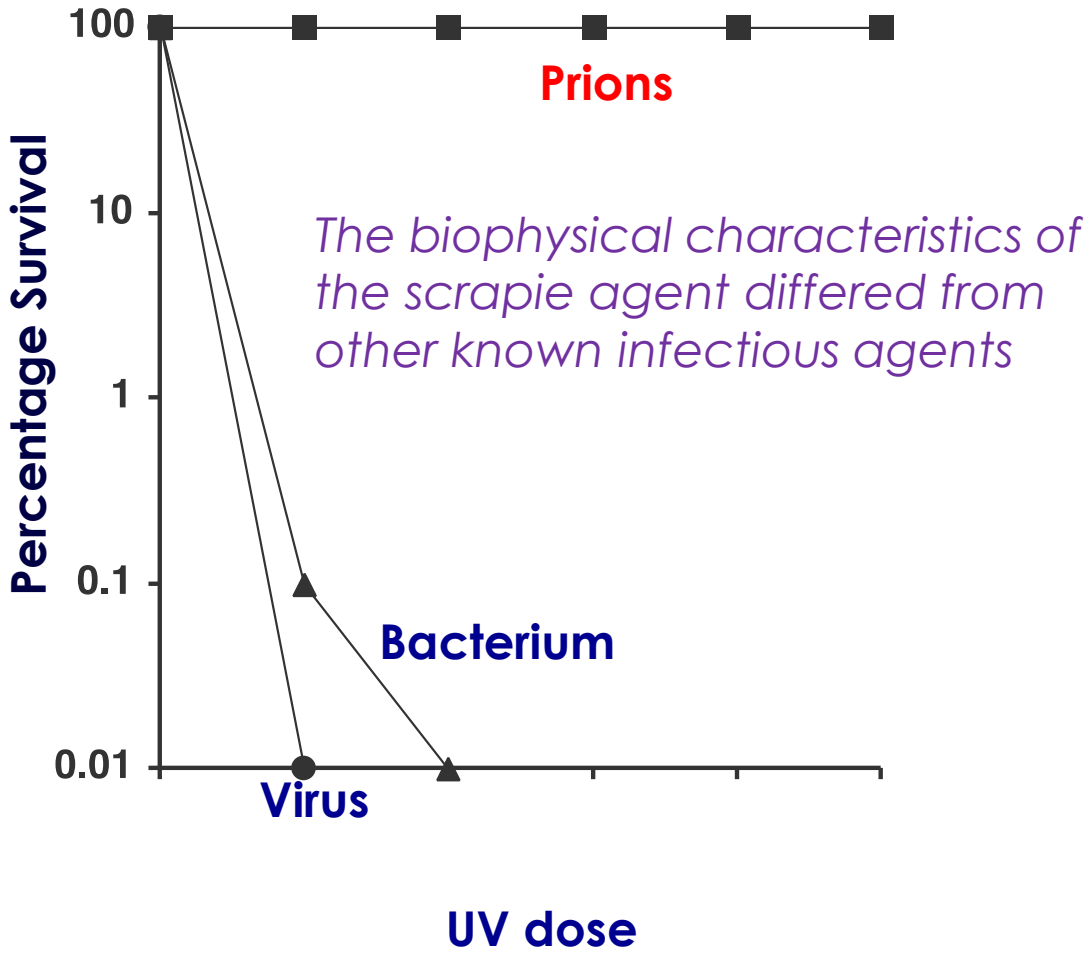
[2]*Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, EHP 441, Papua New Guinea*
[3]*Centre for International Health, ABCRC, Shenton Park Campus, Curtin University, GPO Box U1987, Perth, WA 6845, Australia*

Kuru is so far the principal human epidemic prion disease. While its incidence has steadily declined since the cessation of its route of transmission, endocannibalism, in Papua New Guinea in the 1950s, the arrival of variant Creutzfeldt–Jakob disease (vCJD), also thought to be transmitted by dietary prion exposure, has given kuru a new global relevance. We investigated all suspected cases of kuru from July 1996 to June 2004 and identified 11 kuru patients. There were four females and seven males, with an age range of 46–63 years at the onset of disease, in marked contrast to the age and sex distribution when kuru was first investigated 50 years ago. We obtained detailed histories of residence and exposure to mortuary feasts and performed serial neurological examination and genetic studies where possible. All patients were born a significant period before the mortuary practice of transumption ceased and their estimated incubation periods in some cases exceeded 50 years. The principal clinical features of kuru in the studied patients showed the same progressive cerebellar syndrome that had been previously described. Two patients showed marked cognitive impairment

The scrapie agent lacks nucleic acids

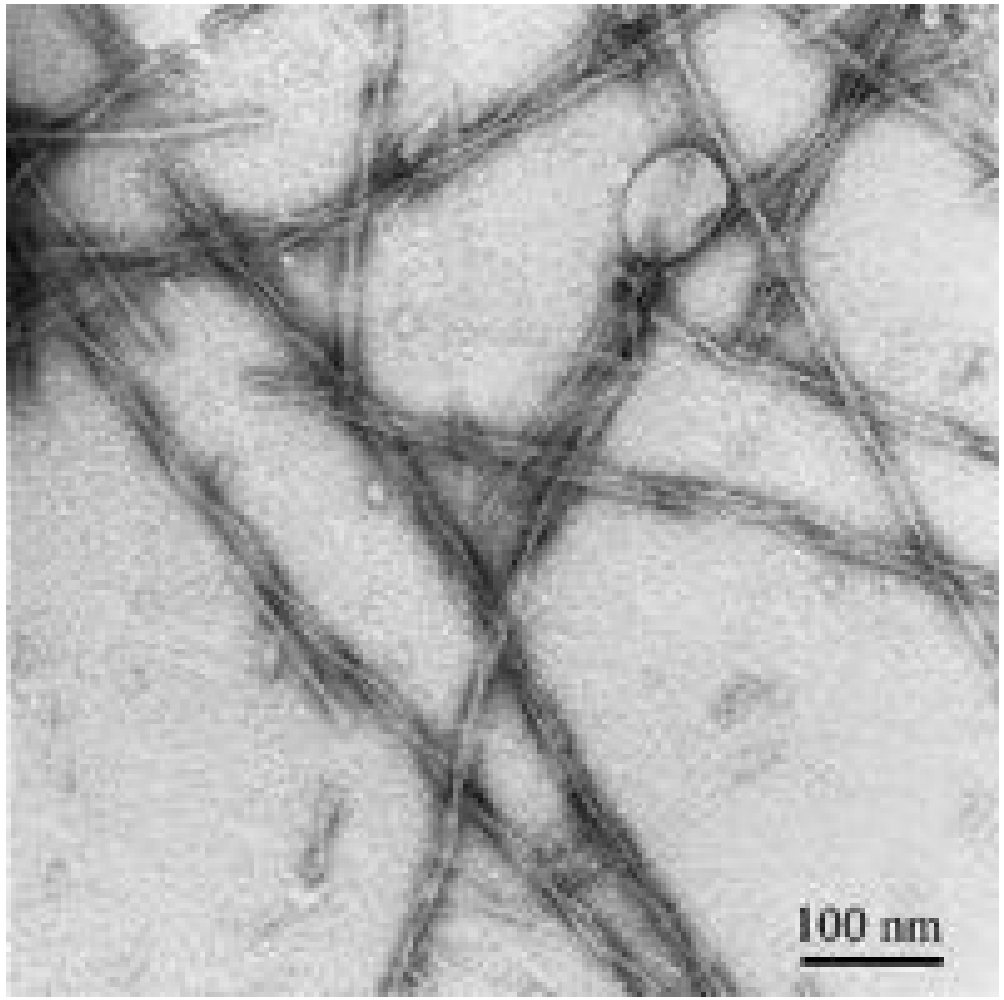
Radiation (non)-inactivation of Scrapie – England 1967



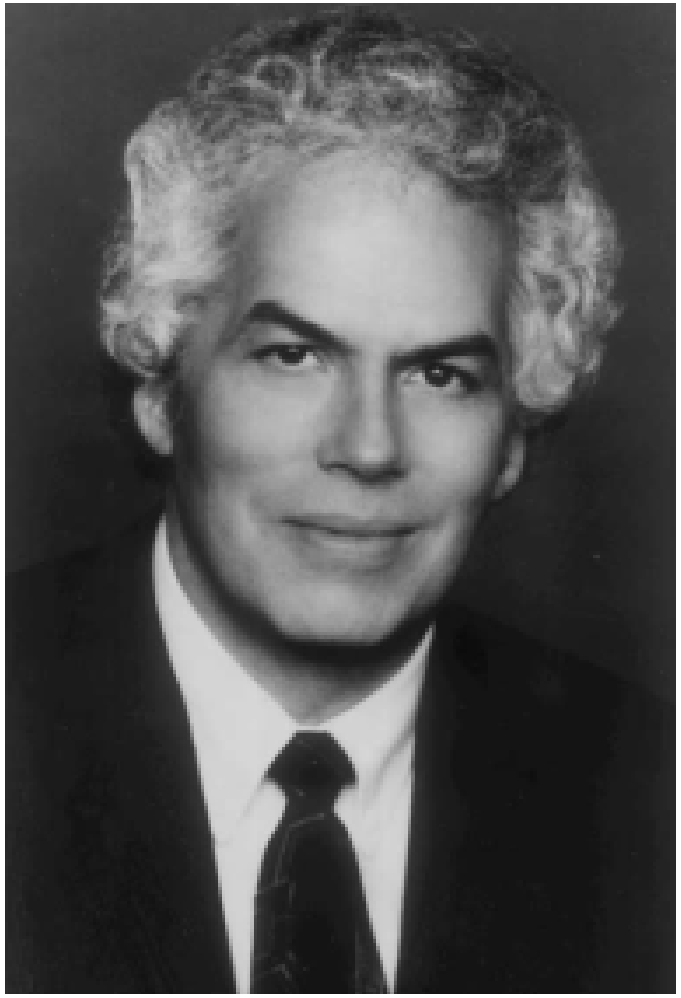
The biophysical characteristics of the scrapie agent differed from other known infectious agents

Tikvah Alper

Purified, highly infectious preparations of scrapie contain amyloid fibrils and no nucleic acids



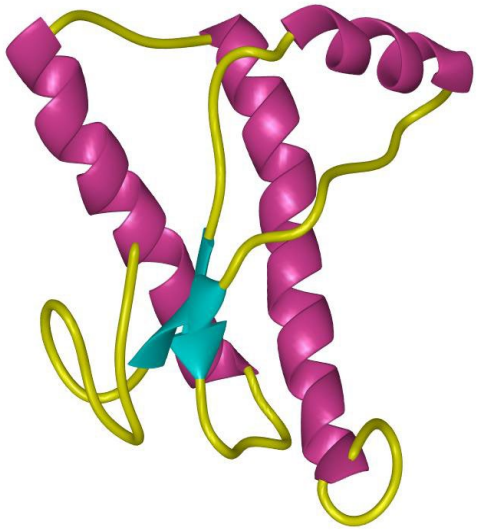
United States 1982



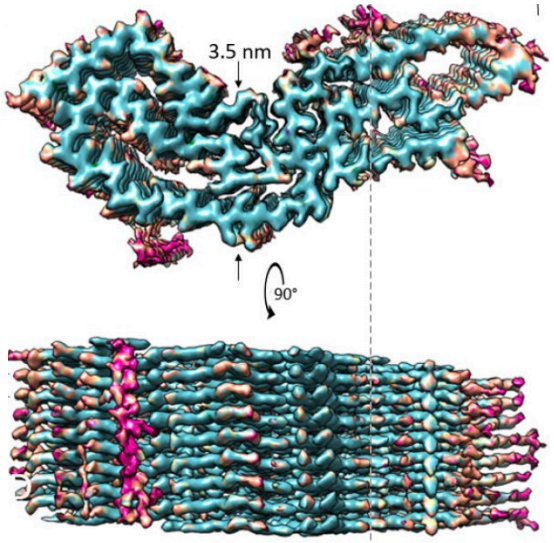
Stanley Prusiner

The prion hypothesis

Normal **PrP^{C}**



Disease **PrP^{Sc}**

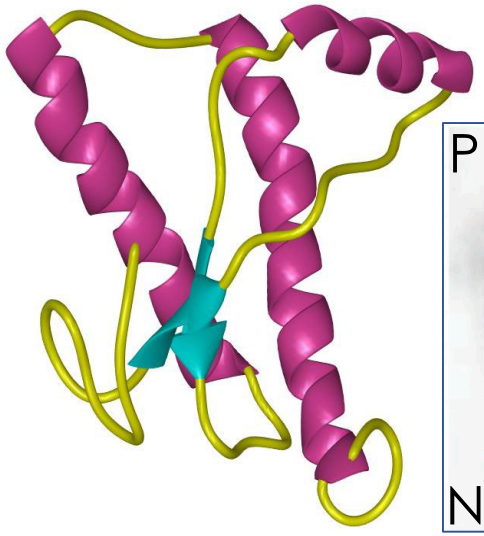


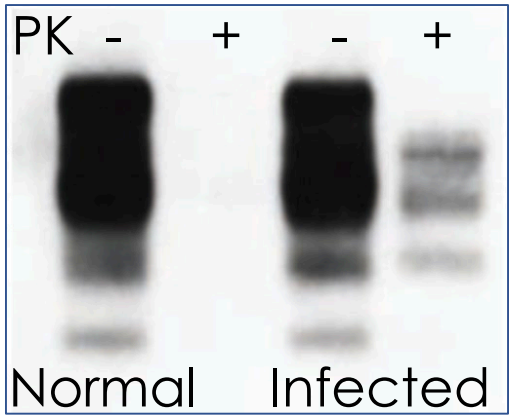
The prion protein exists in two conformations

- The normal, host-encoded form referred to as **PrP^{C}**
- Its infectious counterpart referred to as **PrP^{Sc}**
- Prions lack nucleic acids and are composed of **PrP^{Sc}**

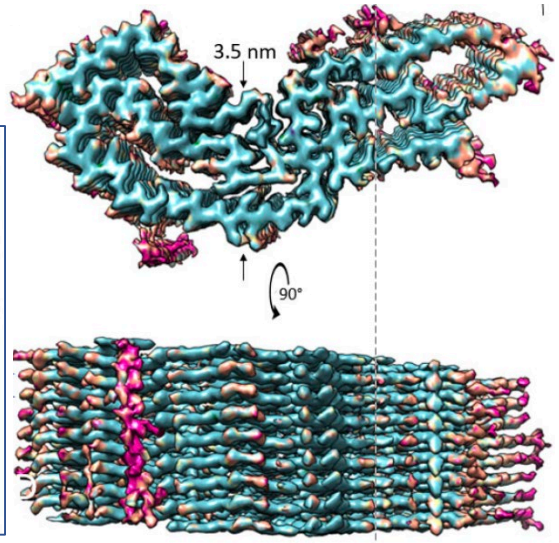
Distinct properties of PrP^{C} and PrP^{Sc}

Normal **PrP^{C}**





Disease **PrP^{Sc}**

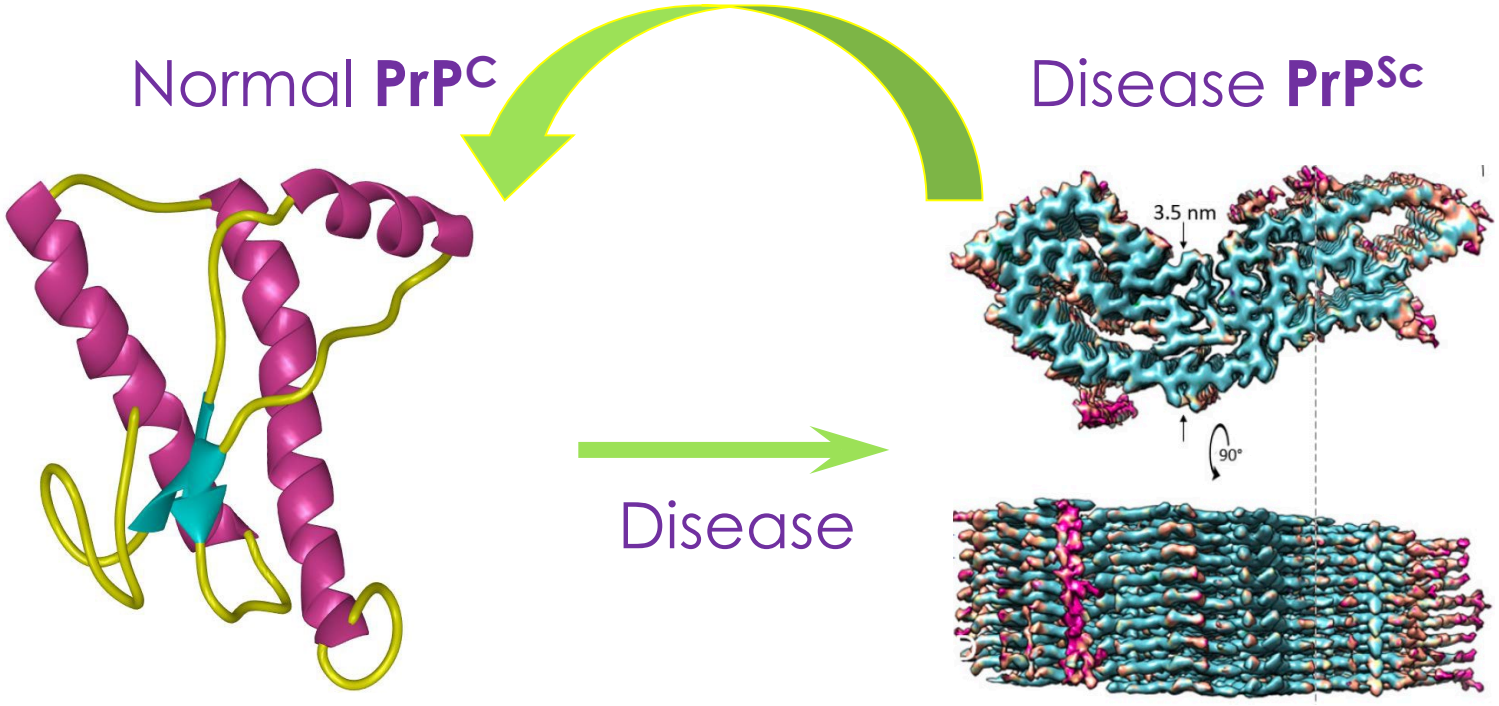


- Not infectious
- Protease-sensitive

- Infectious
- Partially protease-resistant

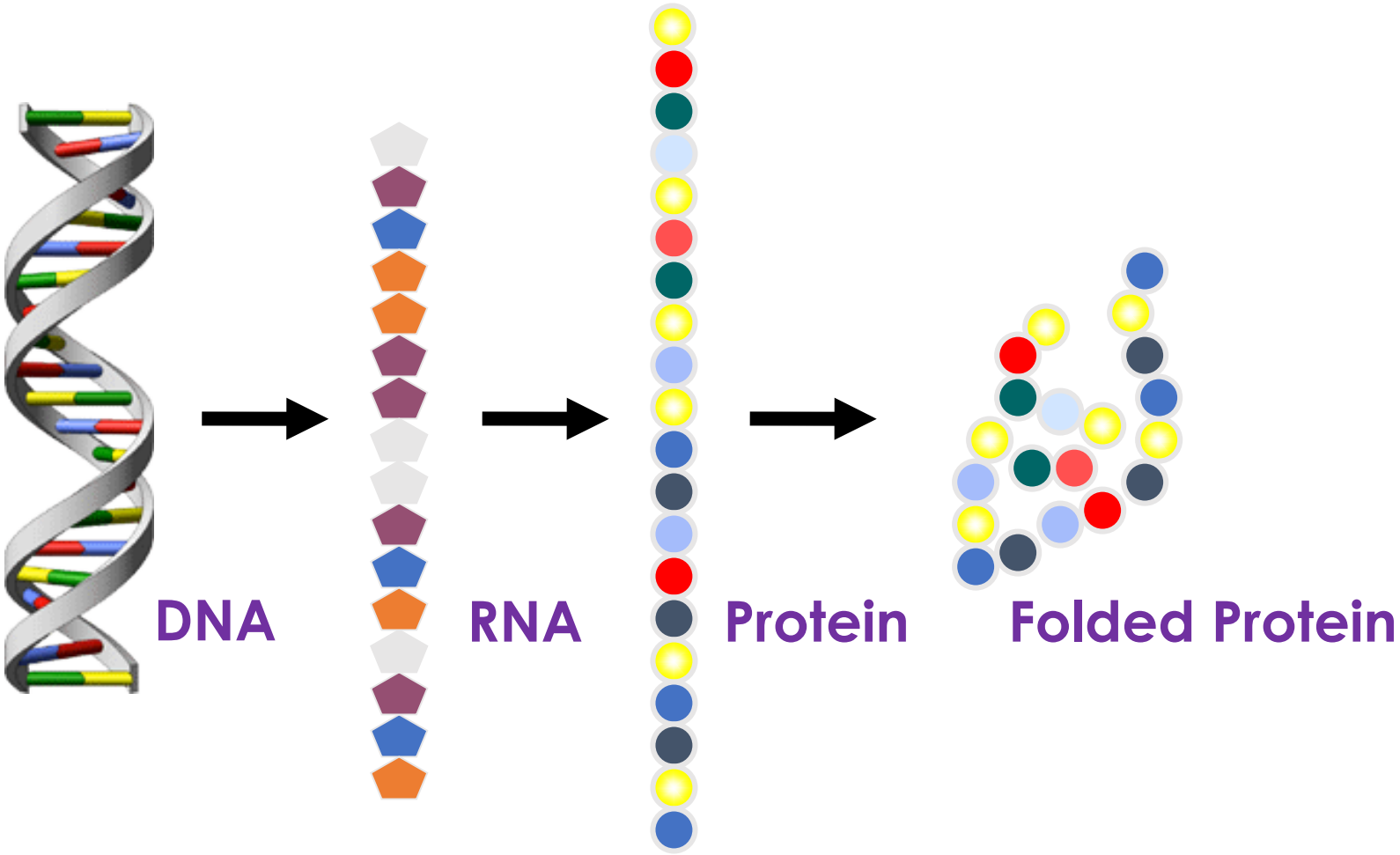
PrP^{C} and **PrP^{Sc}** have different biochemical properties

TSEs are disorders of prion protein conformation



PrP^{Sc} imposes its infectious conformation on **PrP^{C}** resulting in the exponential accumulation of additional **PrP^{Sc}**

The prion hypothesis



The prion hypothesis was heretical since it appeared to contravene the central dogma of molecular biology

Telling Lab - Research Goals

To understand:

- The mechanism of prion propagation
- The molecular basis of prion species barriers
- How prion strains encipher heritable and mutable information in the absence of informational nucleic acids
- The factors controlling how prions evolve and adapt in response to various selective pressures
- The zoonotic risks of emergent prion strains

Abrogating the species barrier to human prions in transgenic mice

Cell, Vol. 83, 79–90, October 6, 1995, Copyright © 1995 by Cell Press

Prion Propagation in Mice Expressing Human and Chimeric PrP Transgenes Implicates the Interaction of Cellular PrP with Another Protein

Glenn C. Telling,* Michael Scott,* James Mastrianni,* Ruth Gabizon,‖ Marilyn Torchia,* Fred E. Cohen,†‡ Stephen J. DeArmond,*§ and Stanley B. Prusiner*†
*Department of Neurology
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‡Department of Cellular and Molecular Pharmacology
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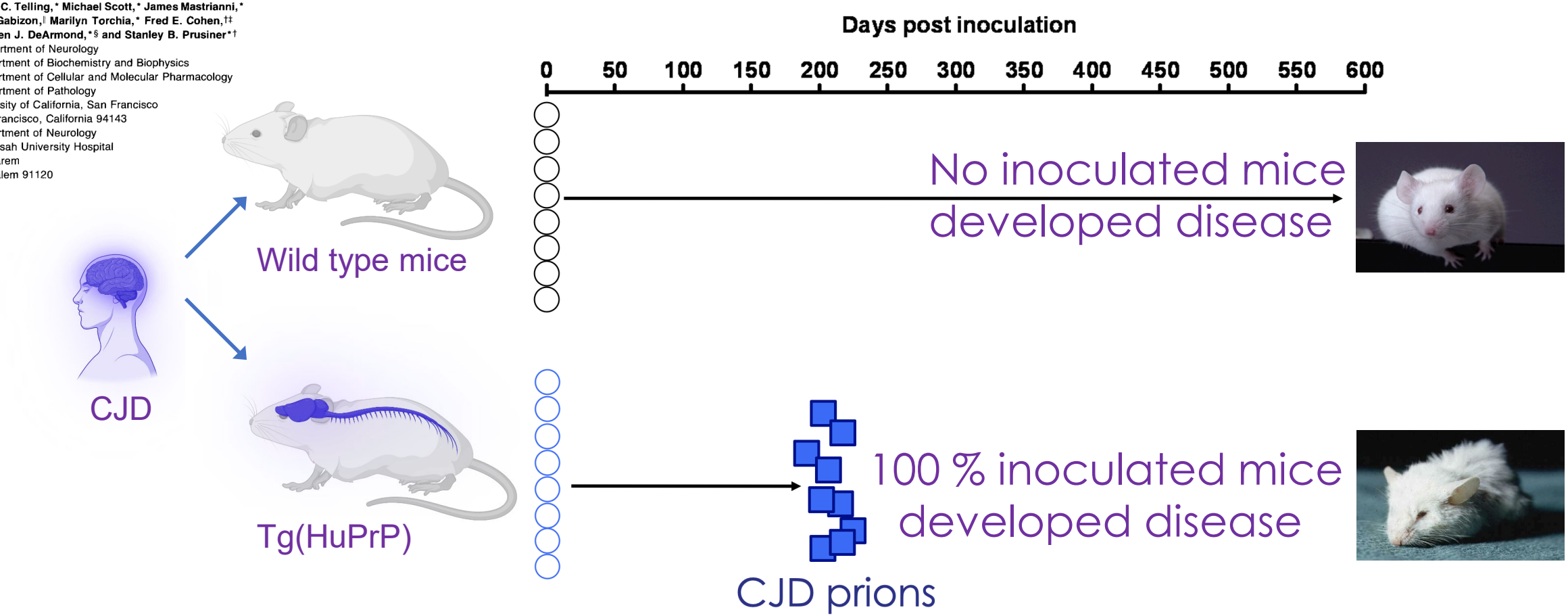
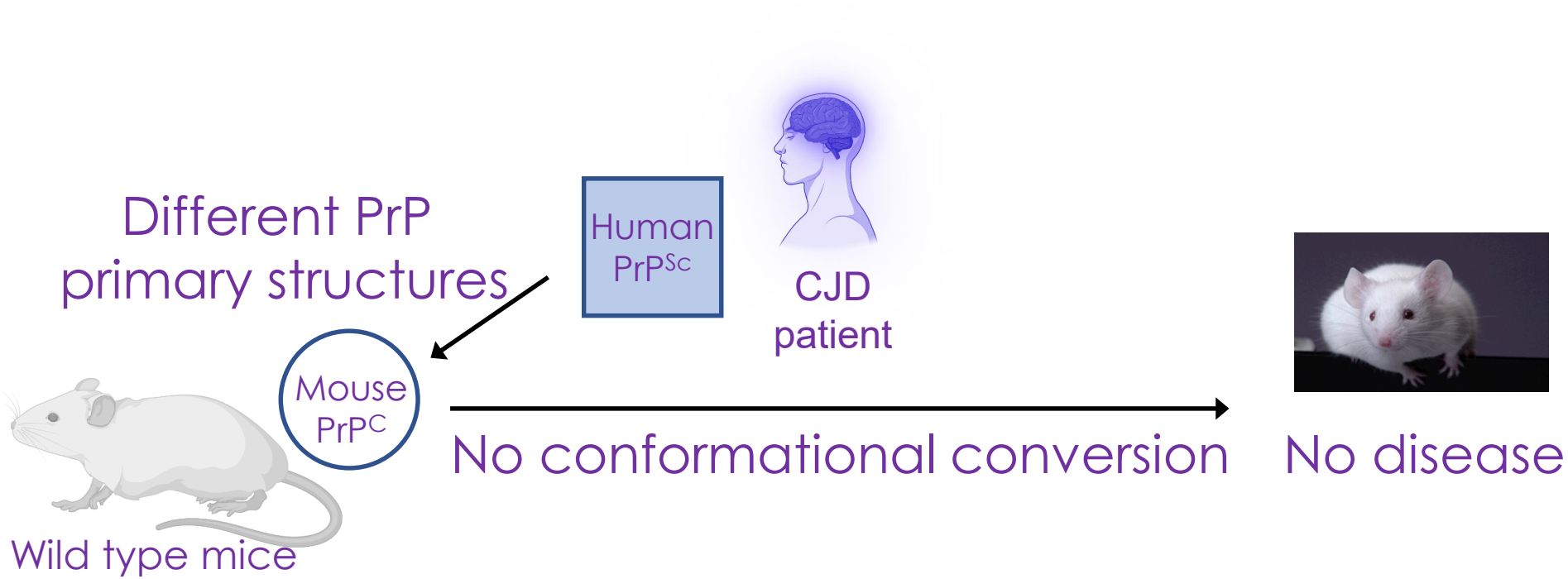
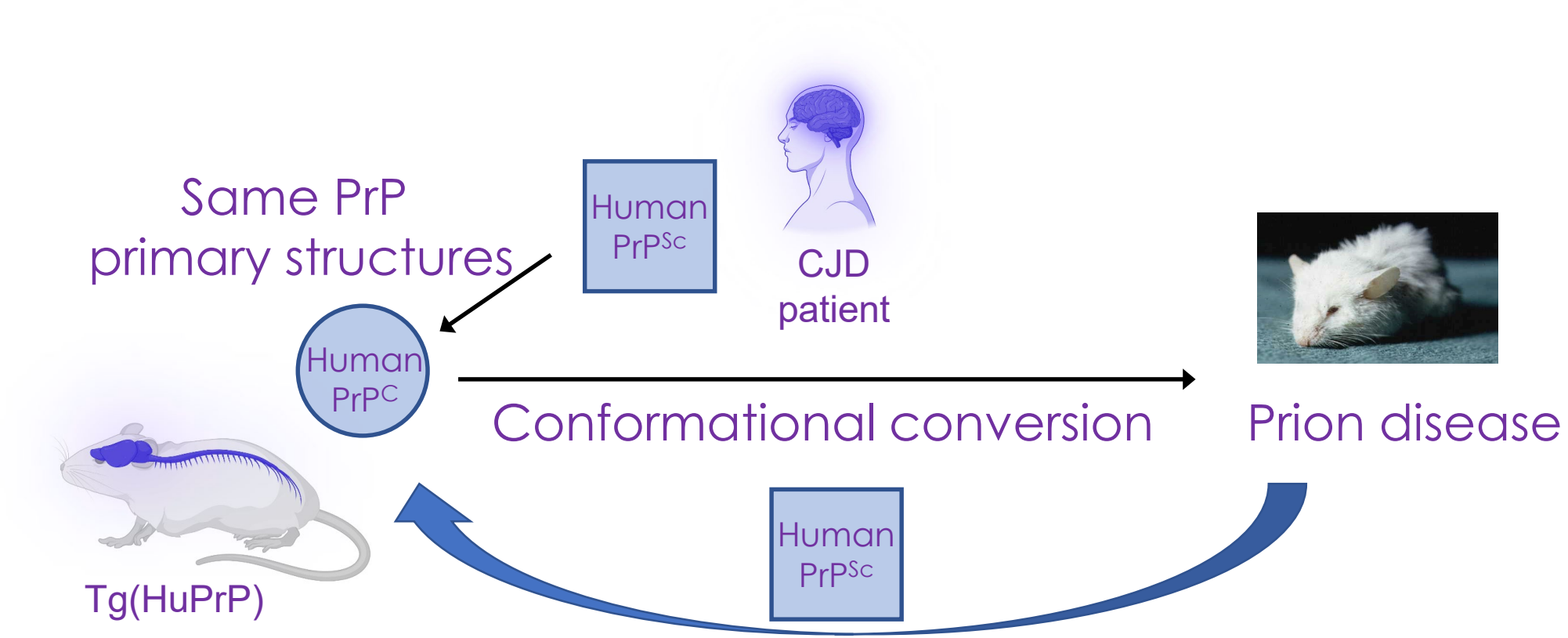
Israel



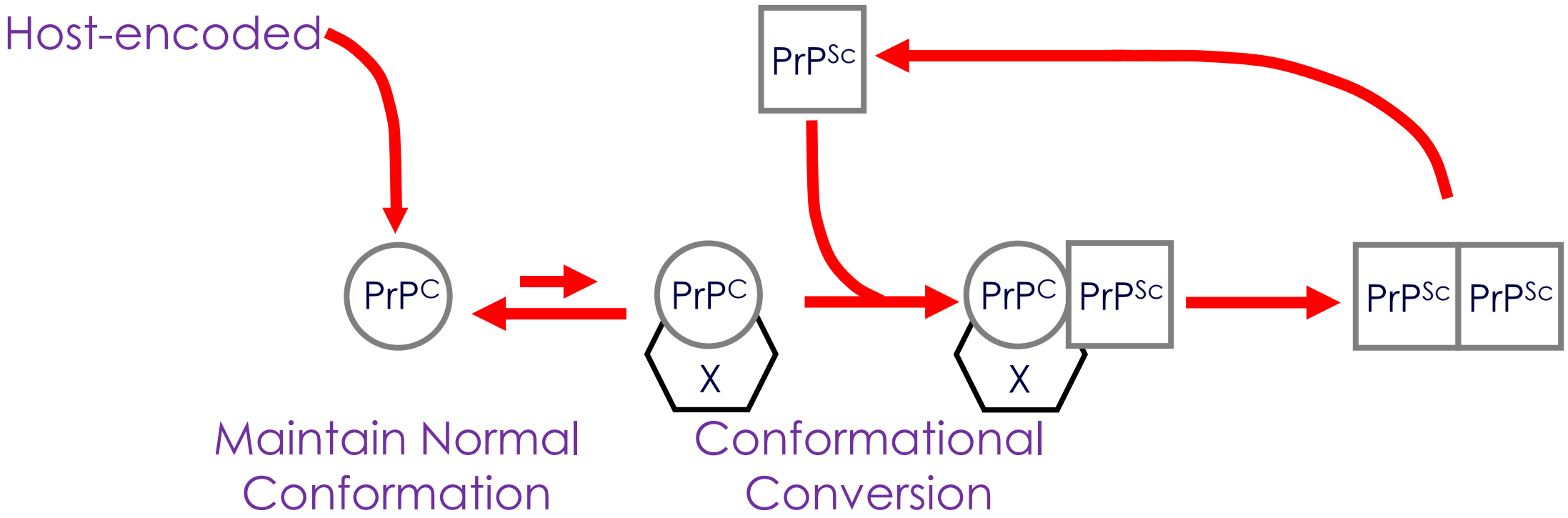
Primary structure of the prion protein influences interspecies prion transmission



Primary structure of the prion protein influences interspecies prion transmission



Prions replicate by template-mediated conformational conversion



How do prions encode heritable strain information in the absence of nucleic acids?

Prion strain properties are inherited on passage from one generation to the next.

How does this occur in the absence of informational nucleic acid in the infectious agent?

How do prions encode heritable strain information in the absence of nucleic acids?

Evidence for the Conformation of the Pathologic Isoform of the Prion Protein Enciphering and Propagating Prion Diversity

Glenn C. Telling, Piero Parchi, Stephen J. DeArmond, Pietro Cortelli, Pasquale Montagna, Ruth Gabizon, James Mastrianni, Elio Lugaresi, Pierluigi Gambetti, Stanley B. Prusiner*

The fundamental event in prion diseases seems to be a conformational change in cellular prion protein (PrP^C) whereby it is converted into the pathologic isoform PrP^{Sc}. In fatal familial insomnia (FFI), the protease-resistant fragment of PrP^{Sc} after deglycosylation has a size of 19 kilodaltons, whereas that from other inherited and sporadic prion diseases is 21 kilodaltons. Extracts from the brains of FFI patients transmitted disease to transgenic mice expressing a chimeric human-mouse PrP gene about 200 days after inoculation and induced formation of the 19-kilodalton PrP^{Sc} fragment, whereas extracts from the brains of familial and sporadic Creutzfeldt-Jakob disease patients produced the 21-kilodalton PrP^{Sc} fragment in these mice. The results presented indicate that the conformation of PrP^{Sc} functions as a template in directing the formation of nascent PrP^{Sc} and suggest a mechanism to explain strains of prions where diversity is encrypted in the conformation of PrP^{Sc}.

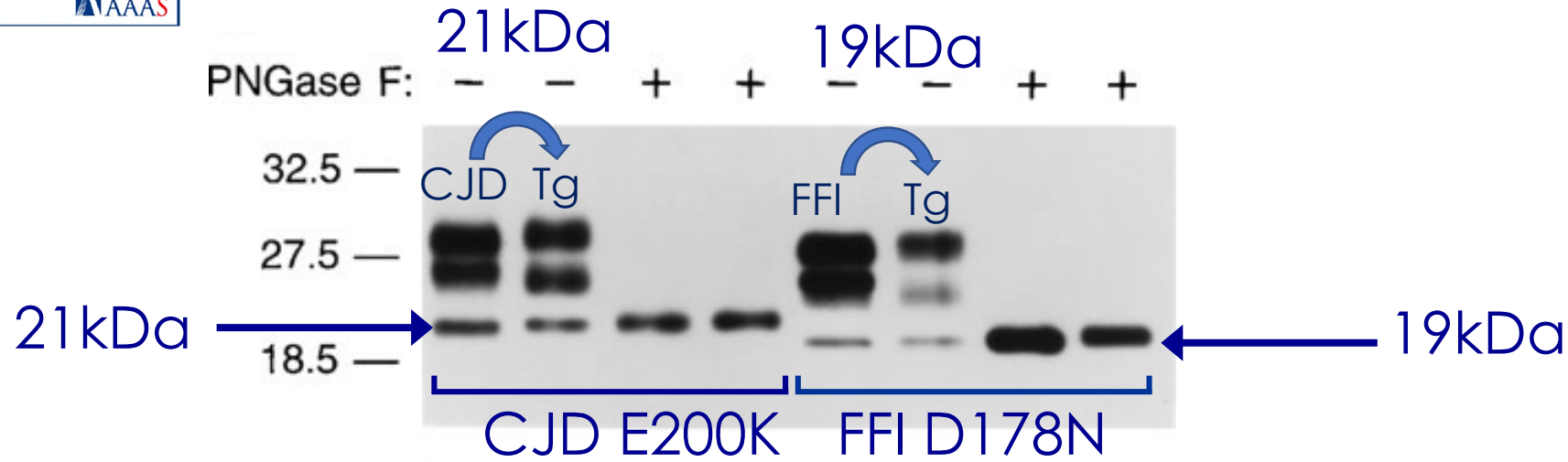
For many years the prion diseases, also called transmissible spongiform encephalopathies, were thought to be caused by slow-acting viruses (*1*), but it is now clear that prions are not viruses and that they are devoid of nucleic acid (*2*, *3*). Prions seem to be composed only of PrP^{Sc} molecules, which are abnormal conformers of a normal, host-encoded protein designated PrP^C (*3*, *4*). PrP^C has a high α-helical content and is virtually devoid of β-sheets, whereas PrP^{Sc} has a high β-sheet content (*4*, *5*); thus, the conversion of PrP^C into PrP^{Sc} involves a profound conformational change. Formation of PrP^{Sc} is a posttranslational process that does not appear to involve a covalent modification of the protein (*6*).

The prion diseases are unique in that they may present as inherited and infectious disorders (*3*, *7*). More than 20 different mutations of the human (Hu) PrP gene segregate with dominantly inherited disease; five of these have been genetically linked to familial Creutzfeldt-Jakob disease (fCJD), Gerstmann-Sträussler-Scheinker disease, and fatal familial insomnia (FFI) (*8*). The most common prion diseases of animals are scrapie of sheep and bovine spongiform encephalopathy; the latter may have been transmitted to people through foods (*9*).

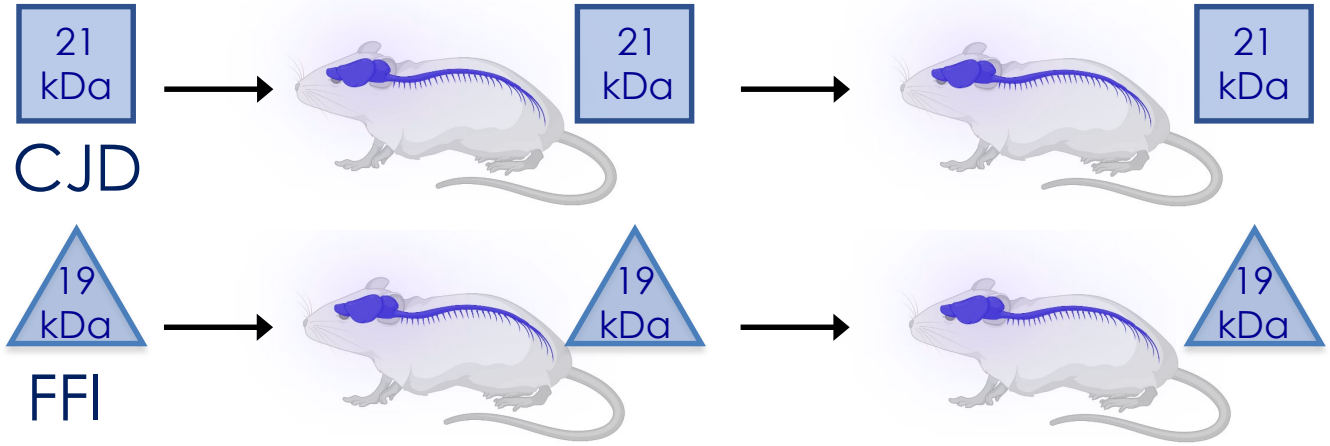
To extend studies on the transmission of

G. C. Telling and J. Mastrianni, Department of Neurology, University of California, San Francisco, CA 94143, USA. P. Parchi and P. Gambetti, Division of Neuropathology, Department of Pathology, Case Western Reserve University, Cleveland, OH 44106, USA. S. J. DeArmond, Departments of Neurology and Pathology, University of California, San Francisco, CA 94143, USA. P. Cortelli, P. Montagna, E. Lugaresi, Department of Neurology, University of Bologna, Bologna 40123, Italy. R. Gabizon, Department of Neurology, Hadassah Medical Center, Hebrew University, Jerusalem 91120, Israel. S. B. Prusiner, Departments of Neurology and Biochemistry and Biophysics, University of California, San Francisco, CA 94143, USA.

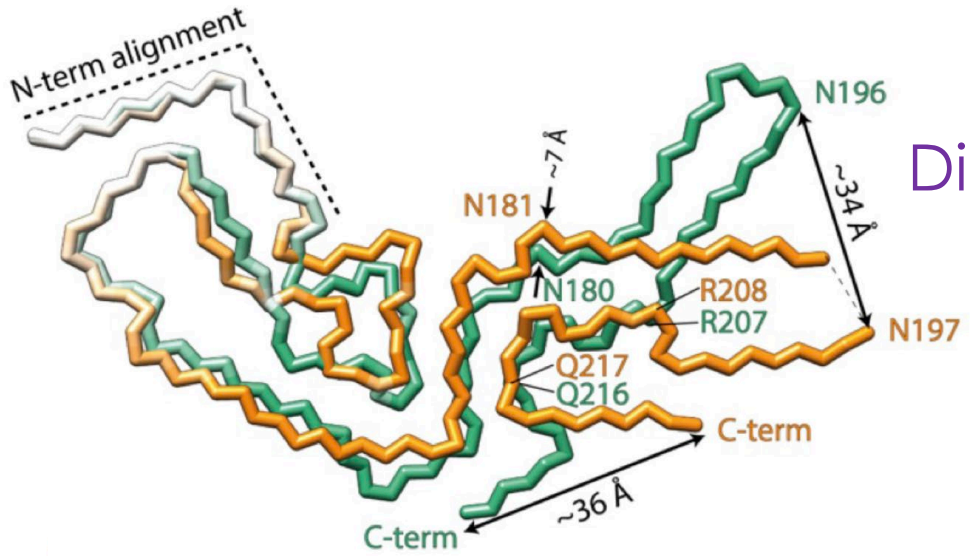
*To whom correspondence should be addressed at the Department of Neurology, HSE-781, University of California, San Francisco, CA 94143-0518, USA.



Strain information is enciphered within distinct prion protein conformations which are faithfully templated during prion replication

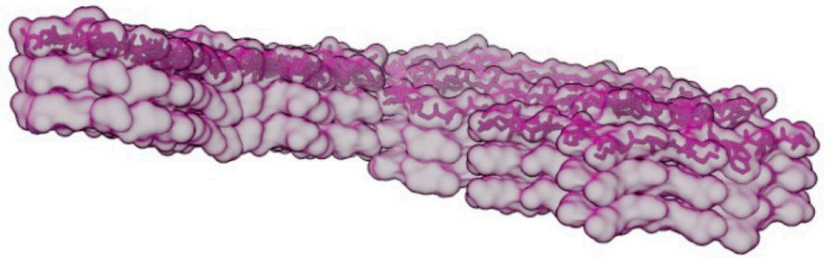


Cryo-EM - Different prion strains are composed of distinct PrP^{Sc} conformers



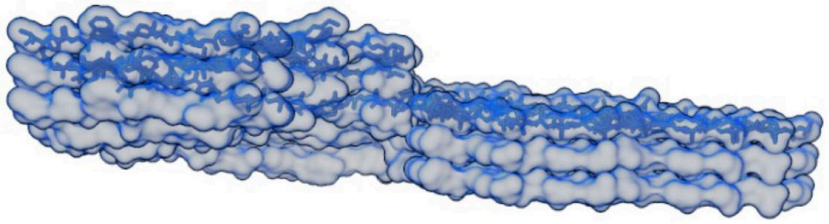
Different monomer conformations

Different stacking of monomers



Mouse RML prions

Different helical pitches of amyloid fibrils



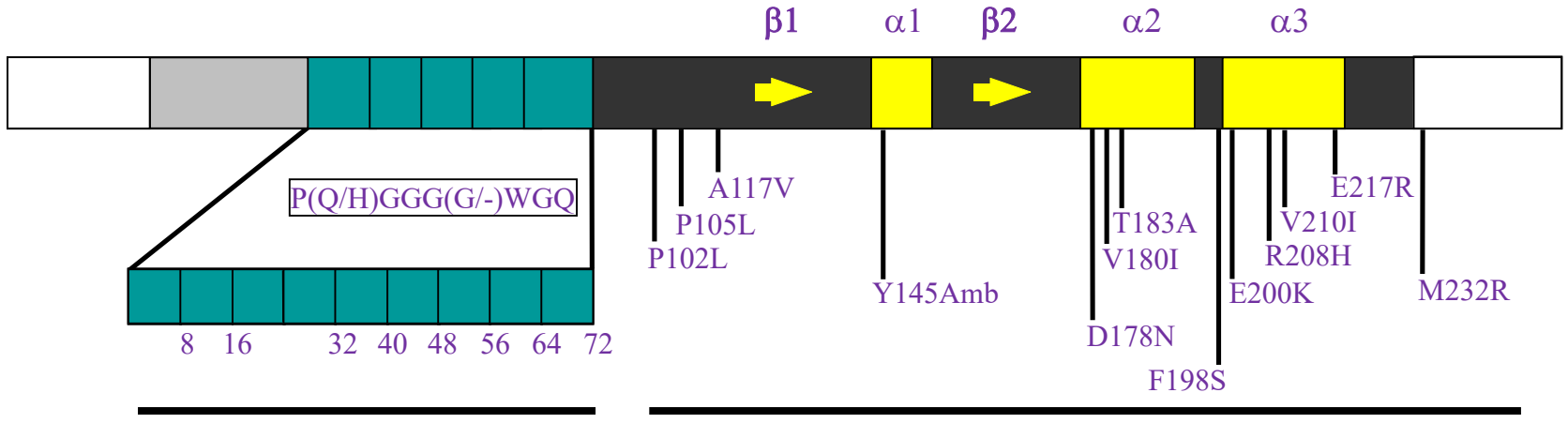
Hamster 263K prions

Inherited human prion diseases

Incidence: 10 – 20 % of all human prion diseases

- Familial CJD
- Gerstmann-Sträussler-Scheinker Syndrome (GSS)
- Fatal Familial Insomnia (FFI)

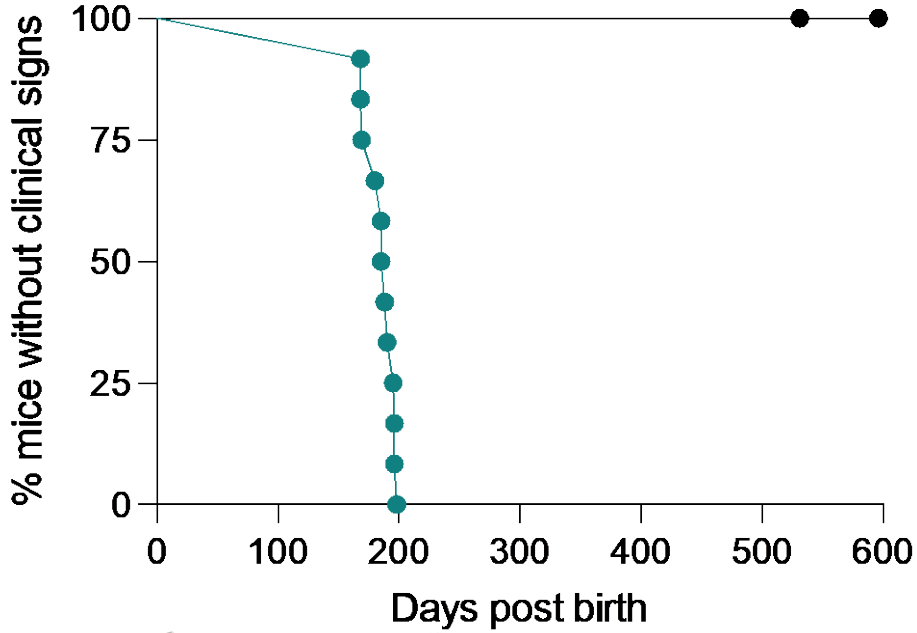
Aetiology: Autosomal dominant inheritance caused by coding sequence mutations in the PrP gene



Octarepeat insertions

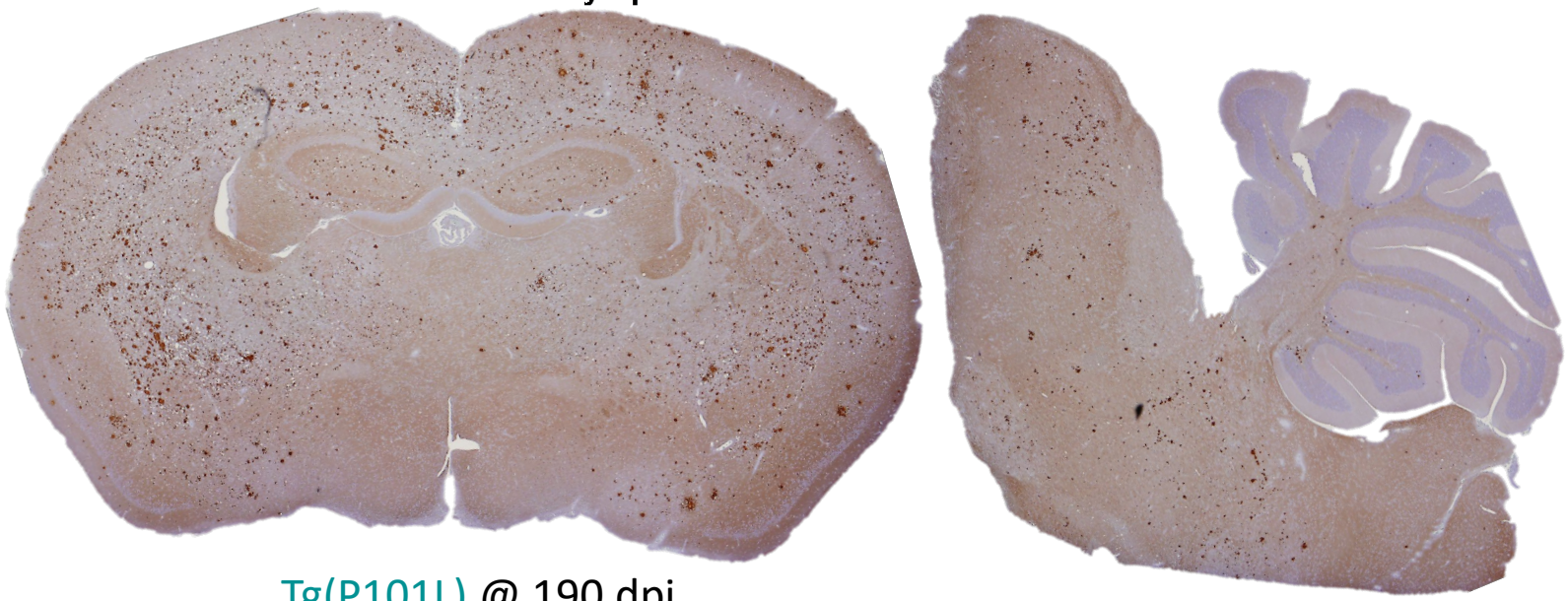
Point mutations

Modelling inherited human prion diseases in Tg mice

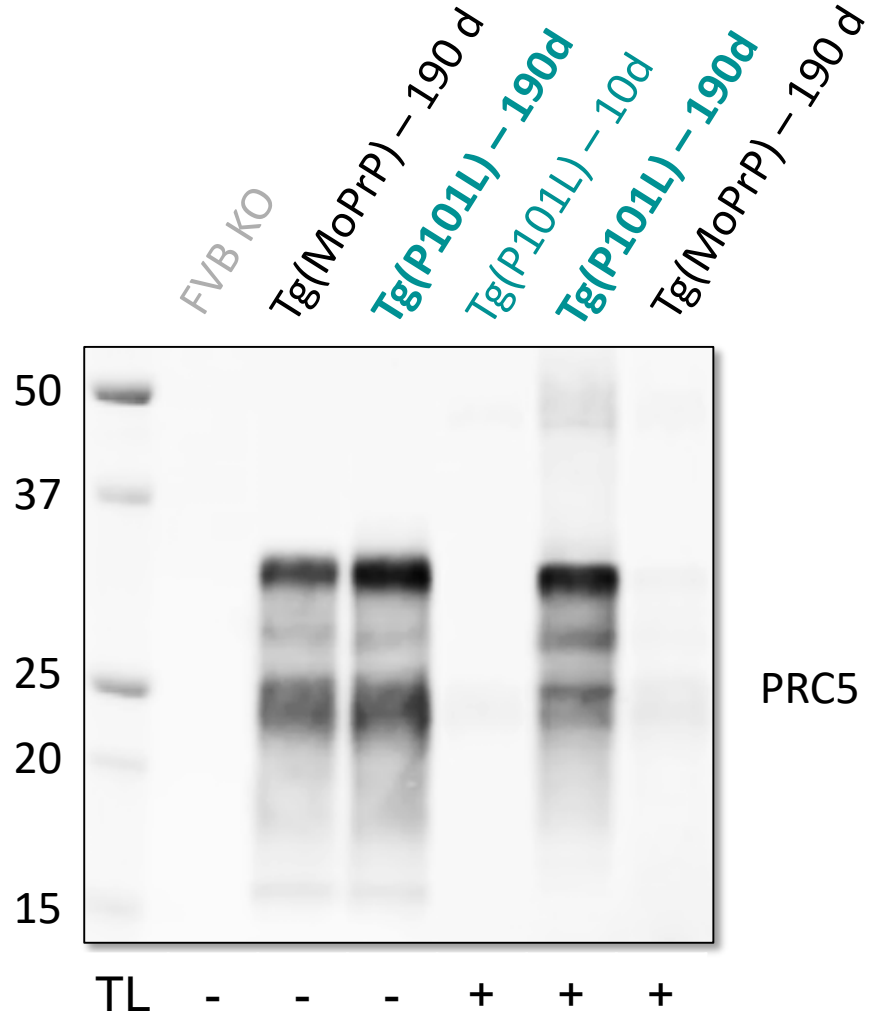


P101L
185±3 (12/12)

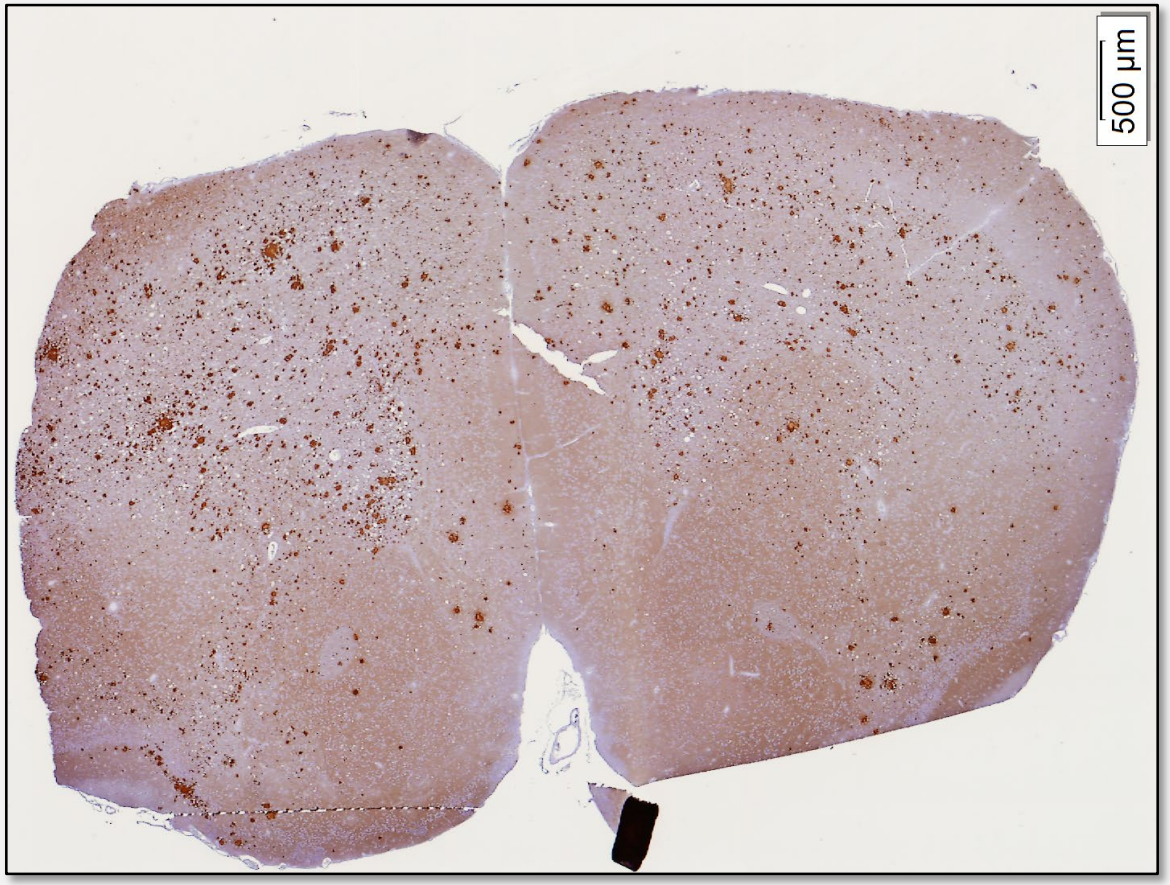
Wild-type
531-596 (0/5)

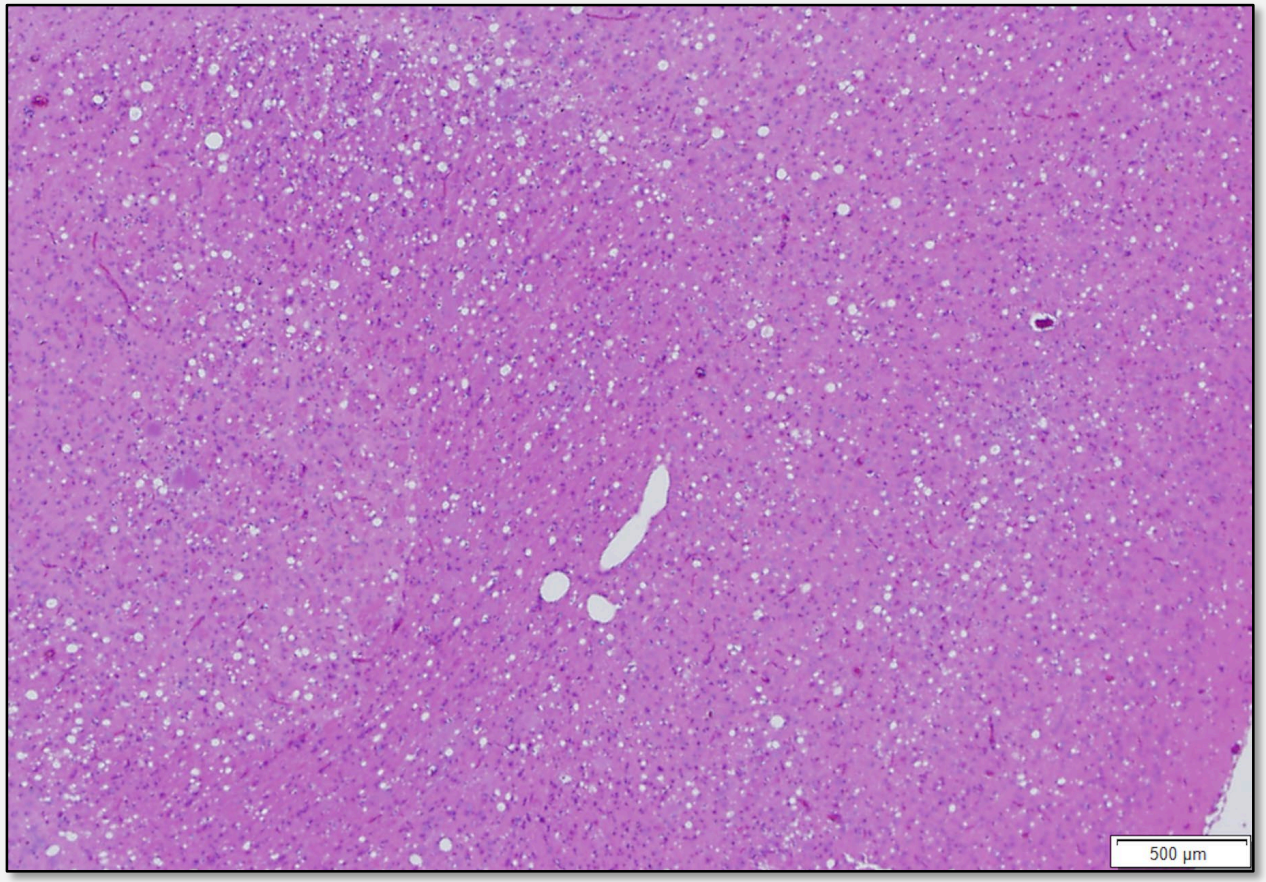


Tg(P101L) @ 190 dpi



Modelling inherited human prion diseases in Tg mice





Transgenic mouse models of prion diseases

NATURAL HOSTS

CJD — Tg(Human PrP)

Scrapie — Tg(Sheep PrP)

BSE — Tg(Cattle PrP)

CWD — Tg(Elk PrP)

CWD — Tg(Deer PrP)

TME — Tg(Mink PrP)

BSE/ CWD — Tg(Cat PrP)

AT RISK SPECIES

Tg(Pig PrP)

Tg(Horse PrP)

EXPERIMENTAL SPECIES

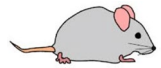
Tg(Mouse PrP)

Tg(Bank Vole PrP)

POLYMORPHISMS

Tg(DeerPrP-S96)

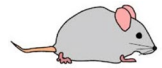
Tg(DeerPrP-H95)



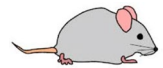
Tg(DeerPrP-F225)

Tg(HumanPrP-V129)

Tg(SheepPrP-A136)



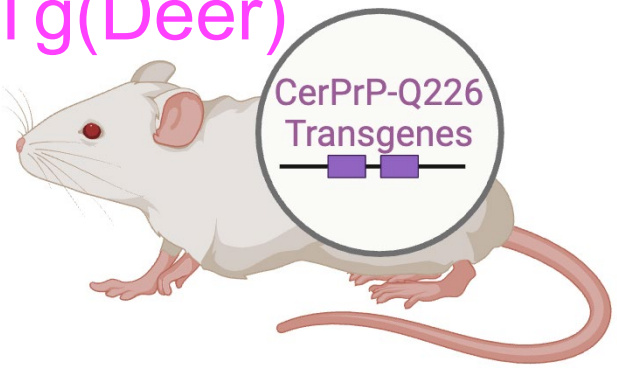
Tg(ElkPrP-L132)

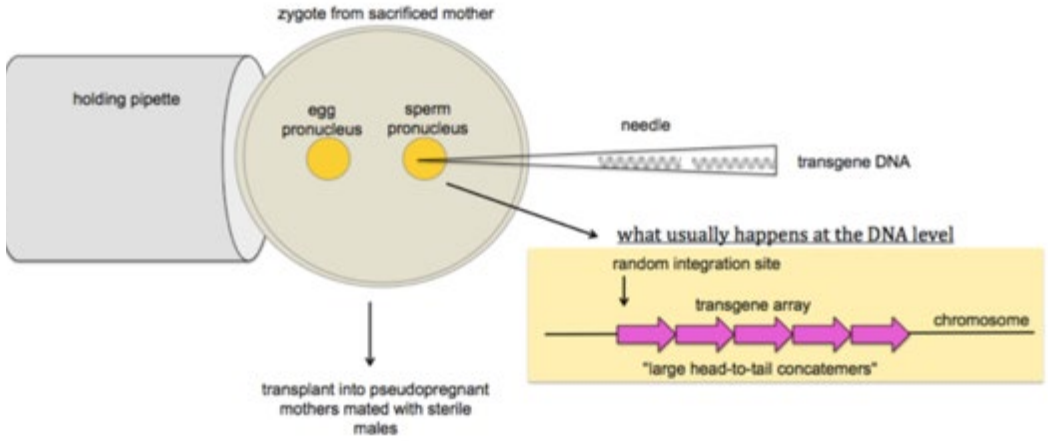


Tg(Cattle-K211PrP)

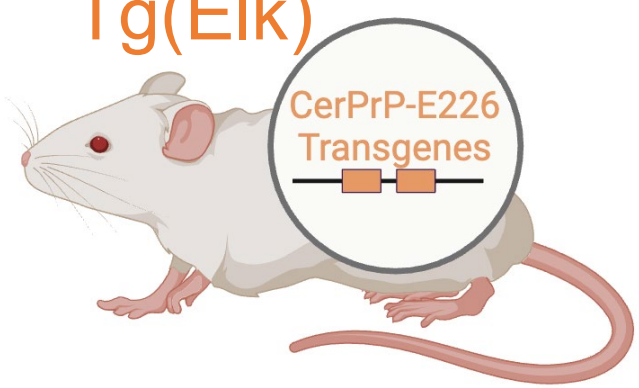
Drawbacks of conventional transgenic mice

Tg(Deer)





Tg(Elk)



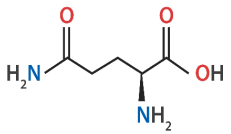
- Uncontrolled transgene copy number and chromosomal integration
- Uncontrolled transgene expression
- Different expression vectors
- Challenging to assess the effects of PrP allele heterozygosity
- Uncertain peripheral expression

North American deer and elk PrP differ at residue 226

North American deer

PRNP

Codon 226 CAG

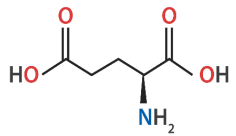


Glutamine (Q)

North American elk

PRNP

Codon 226 GAG

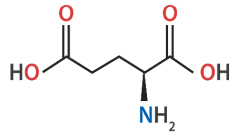


Glutamate (E)

Gene targeted (Gt) mice expressing CerPrP^{C}-E226 (elk) and CerPrP^{C}-Q226 (deer)

GtE

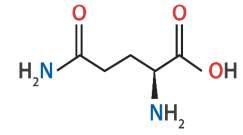
Codon 226



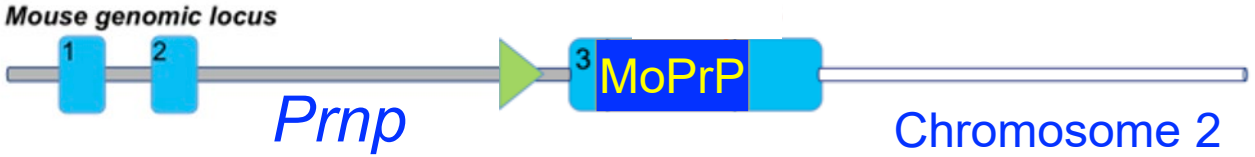
Glutamate (E)

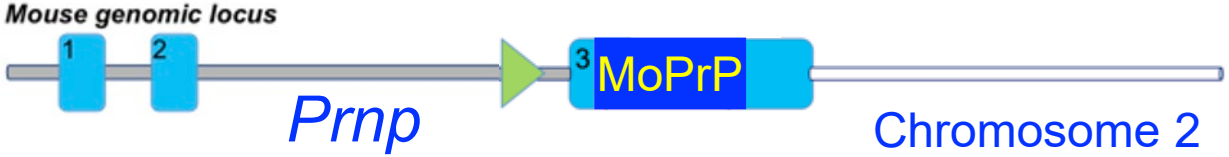
GtQ

Codon 226



Glutamine (Q)





PNAS

Primary structural differences at residue 226 of deer and elk PrP dictate selection of distinct CWD prion strains in gene-targeted mice

Jifeng Bian[a,b,1], Jeffrey R. Christiansen[a,b,1], Julie A. Moreno[a,b], Sarah J. Kane[a,b], Vadim Khaychuk[a,b], Joseph Gallegos[a,b], Sehun Kim[a,b], and Glenn C. Telling[a,b,2]

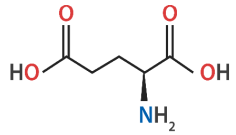
[a]Prion Research Center, Colorado State University, Fort Collins, CO 80525; and [b]Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO 80525

Bian et al., *PNAS*, 2019

Gene targeted (Gt) mice expressing CerPrP^{C}-E226 (elk) and CerPrP^{C}-Q226 (deer)

GtE

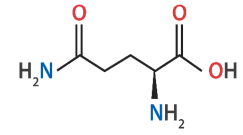
Codon 226



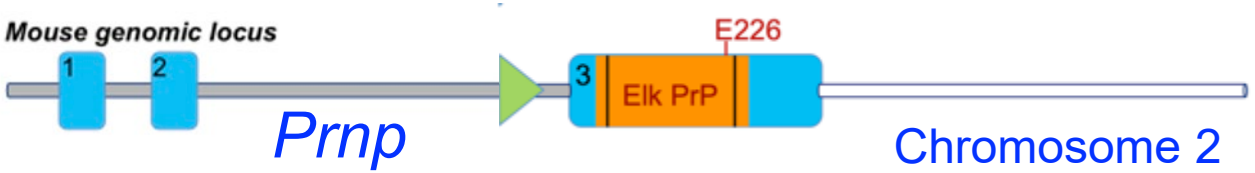
Glutamate (E)

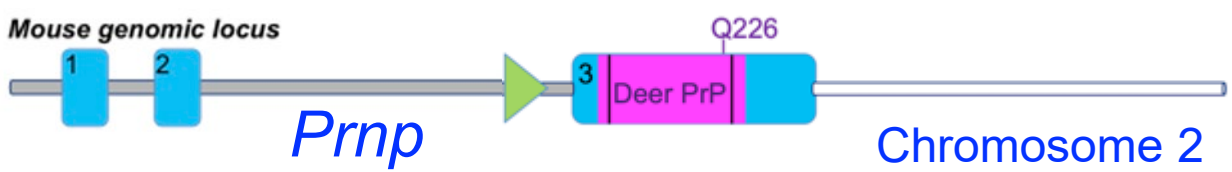
GtQ

Codon 226



Glutamine (Q)





PNAS

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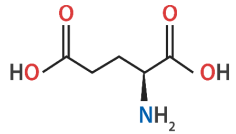
Bian et al., *PNAS*, 2019

Gene targeted (Gt) mice expressing CerPrP^{C}-E226 (elk) and CerPrP^{C}-Q226 (deer)

GtE

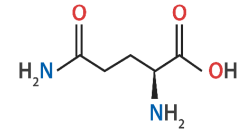
Codon 226



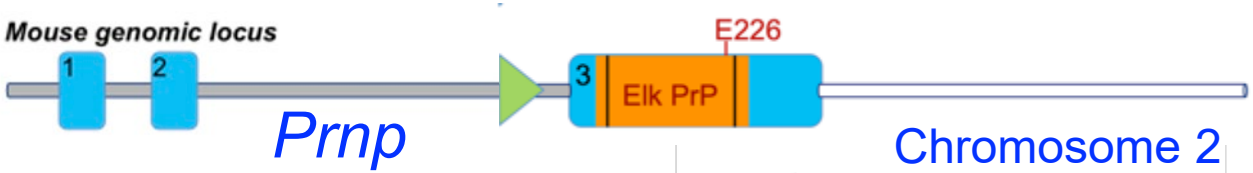
Glutamate (E)

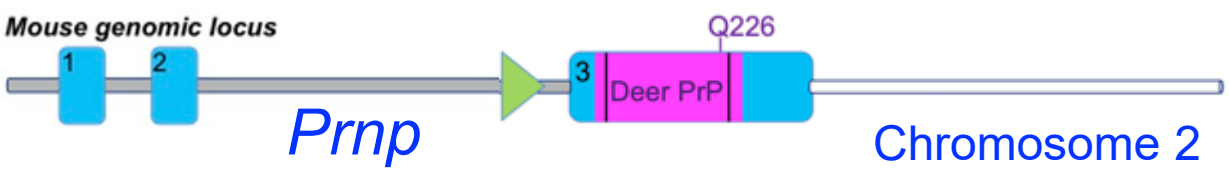
GtQ

Codon 226

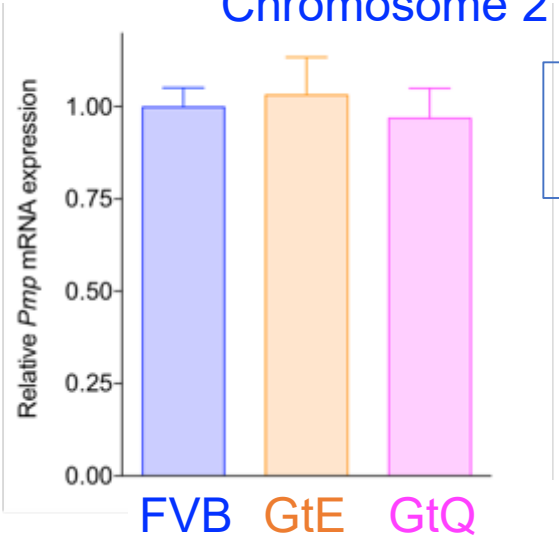


Glutamine (Q)

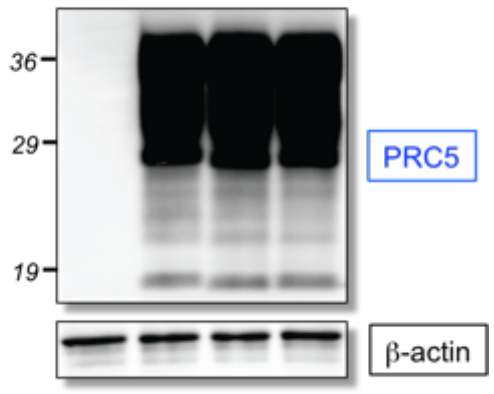




CNS mRNA



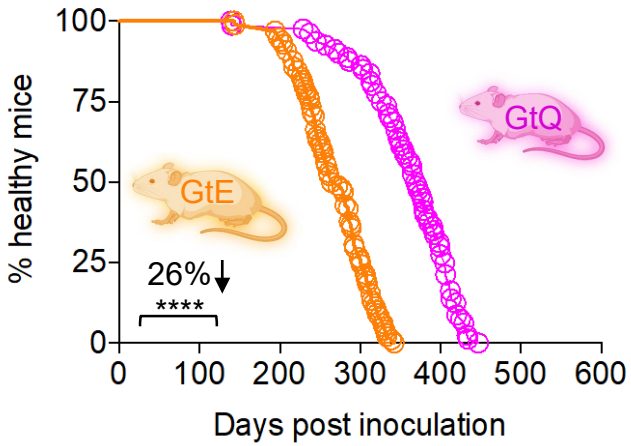
CNS PrP

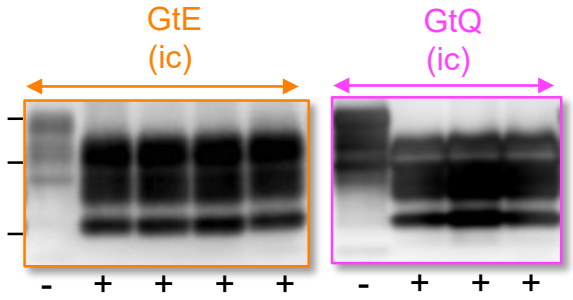


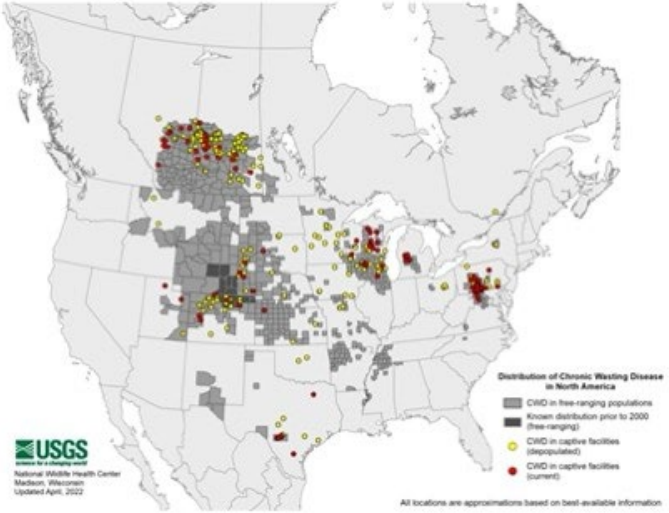
Bian et al., *PNAS*, 2019

Variation at residue 226 influences CWD prion disease kinetics in Gt mice

Disease onset - consistently more rapid in GtE than GtQ



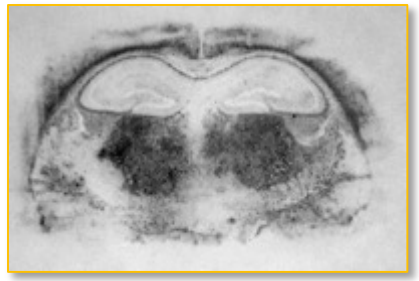


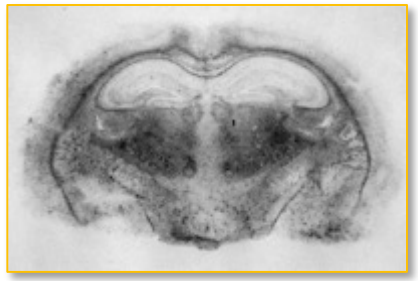


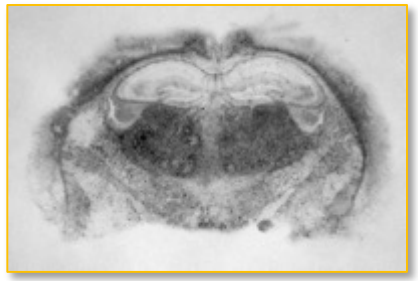
We ascribe this difference to residue 226 since Gt mice are otherwise genetically identical

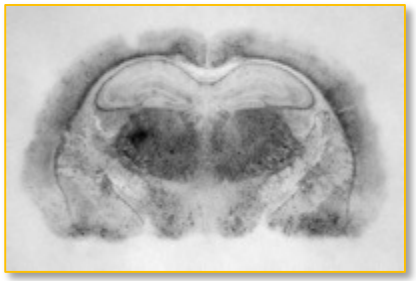
Bian et al., *PNAS*, 2019

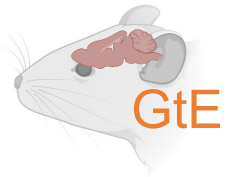
Residue 226 affects CNS targeting of CWD prions


Elk 99W12389 - GtE

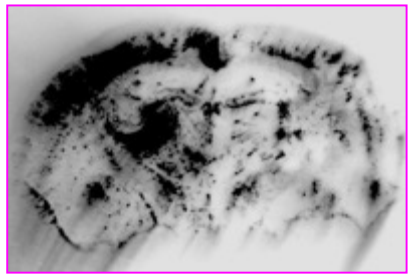

Elk Bala-04 - GtE

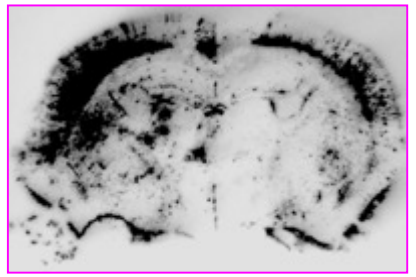

Elk 012-09442 - GtE

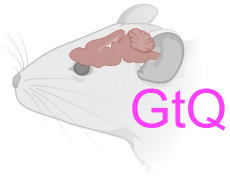

Elk Bala-01 - GtE



Diffuse and symmetrical pattern of PrP^{Sc} distribution in GtE mice infected with North American elk CWD.


Elk 99W12389 - GtQ


Deer D10 - GtQ



Disorganized and asymmetrical pattern of PrP^{Sc} distribution in GtQ mice.

Bian et al., *PNAS*, 2019

Novel emergent CWD strains in Europe

PLOS PATHOGENS

RESEARCH ARTICLE

Adaptive selection of a prion strain conformer corresponding to established North American CWD during propagation of novel emergent Norwegian strains in mice expressing elk or deer prion protein

Jifeng Bian[1], Sehun Kim[1], Sarah J. Kane[1], Jenna Crowell[1], Julianna L. Sun[1,2], Jeffrey Christiansen[1], Eri Saijo[1], Julie A. Moreno[1], James DiLisio[1], Emily Burnett[1], Sandra Pritzkow[3], Damian Gorski[3], Claudio Soto[3], Terry J. Kreeger[4], Aru Balachandran[5], Gordon Mitchell[5], Michael W. Miller[6], Romolo Nonno[7], Turid Vikøren[8], Jørn Våge[8], Knut Madslien[8], Linh Tran[8], Tram Thu Vuong[8], Sylvie L. Benestad[8], Glenn C. Telling[1,2]*

1 Prion Research Center (PRC), the Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, Colorado, United States of America, **2** Program in Cell and Molecular Biology, Colorado State University, Fort Collins, Colorado, United States of America, **3** Mitchell Center for Alzheimer's Disease and Related Brain Disorders, Department of Neurology, University of Texas Houston Medical School, Houston, Texas, United States of America, **4** Wyoming Game and Fish Department, Wheatland, Wyoming, United States of America, **5** Canadian Food Inspection Agency, National and OIE Reference Laboratory for Scrapie and CWD, Ottawa, Canada, **6** Colorado Parks and Wildlife, Fort Collins, Colorado, United States of America, **7** Istituto Superiore di Sanità, Department of Veterinary Public Health, Nutrition and Food Safety, Rome, Italy, **8** Norwegian Veterinary Institute, OIE Reference laboratory for CWD, Oslo, Norway

* glenn.telling@colostate.edu

OPEN ACCESS

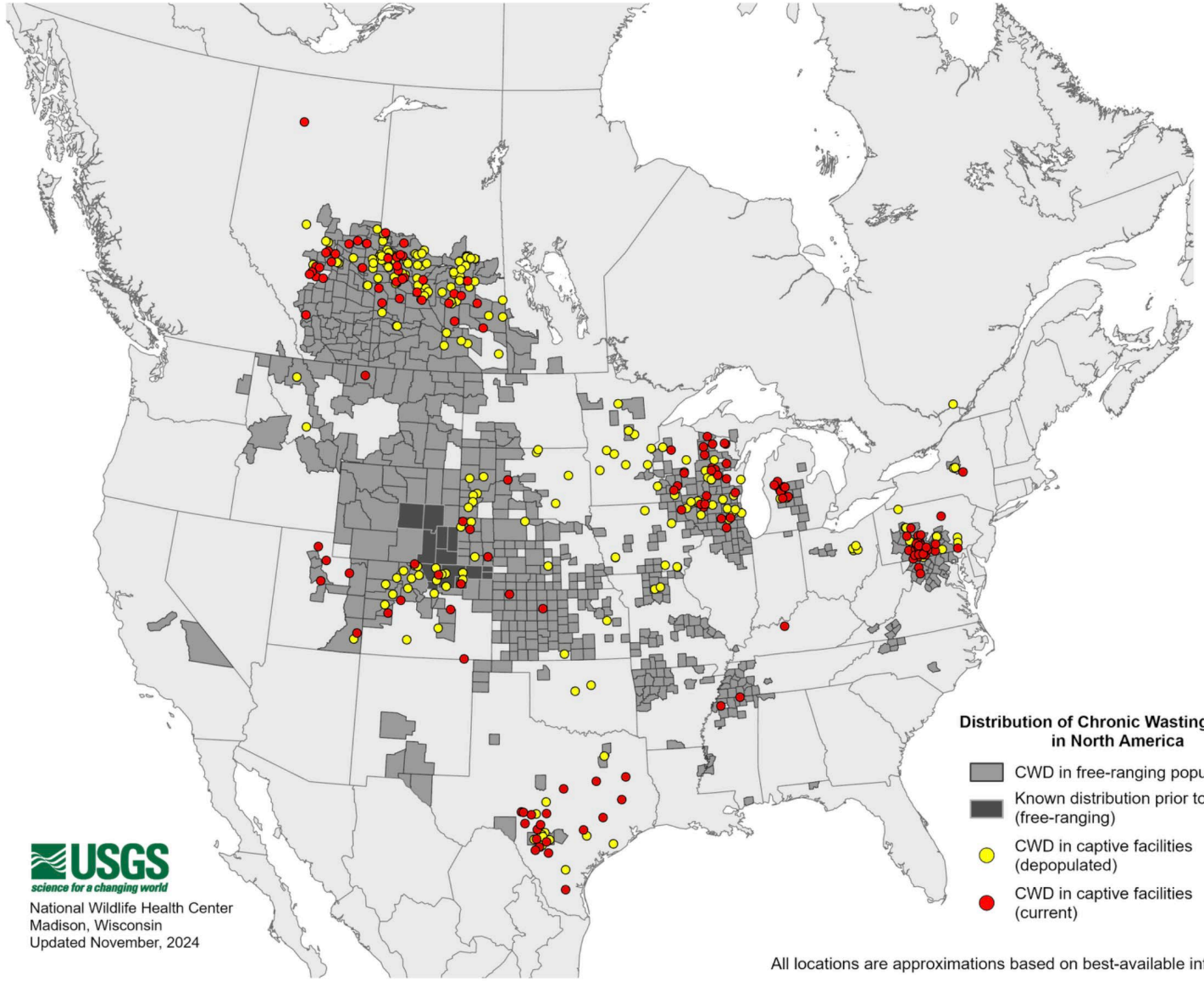
Citation: Bian J, Kim S, Kane SJ, Crowell J, Sun JL, Christiansen J, et al. (2021) Adaptive selection of a prion strain conformer corresponding to established North American CWD during propagation of novel emergent Norwegian strains in mice expressing elk or deer prion protein. PLoS Pathog 17(7): e1009748. https://doi.org/10.1371/journal.ppat.1009748

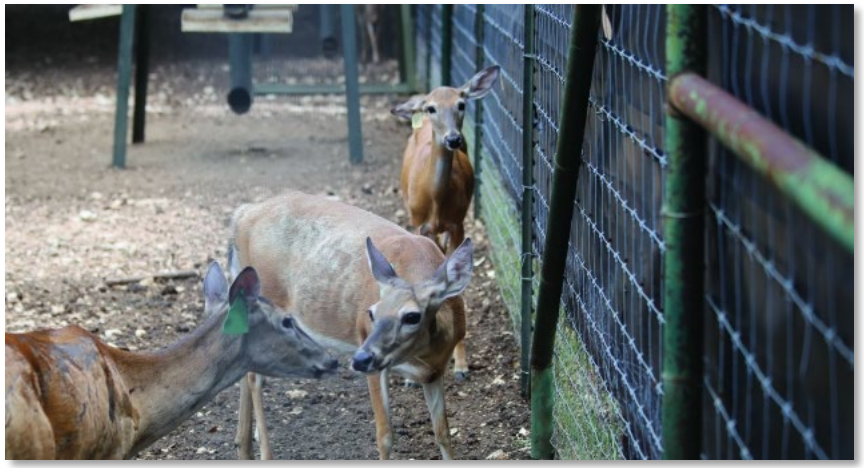
Novel Prion Strain as Cause of Chronic Wasting Disease in a Moose, Finland

Julianna L. Sun, Sehun Kim, Jenna Crowell, Bailey K. Webster, Emma K. Raisley, Diana C. Lowe, Jifeng Bian, Sirkka-Liisa Korpenfelt, Sylvie L. Benestad, Glenn C. Telling

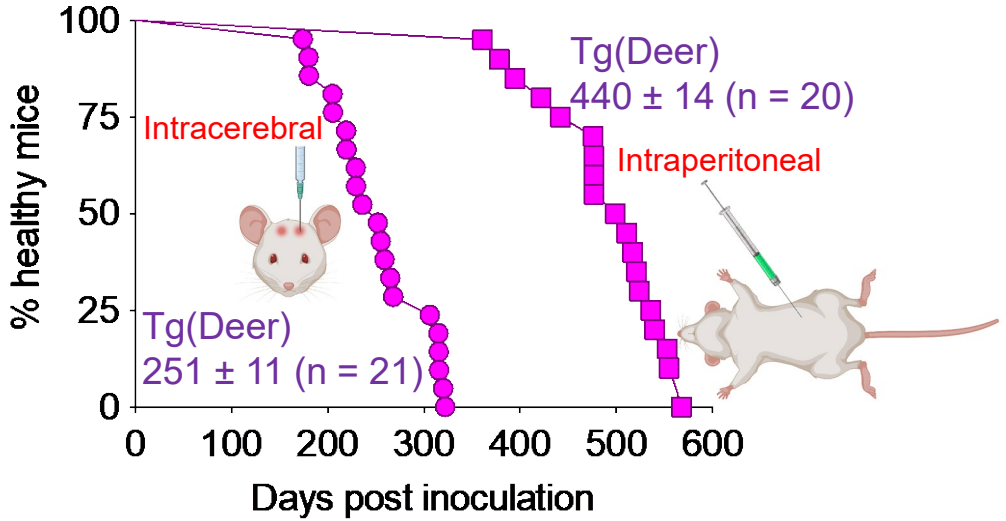
Emerging Infectious Diseases, 2023

Naturally contagious CWD transmission linked to efficient prion propagation by peripheral routes



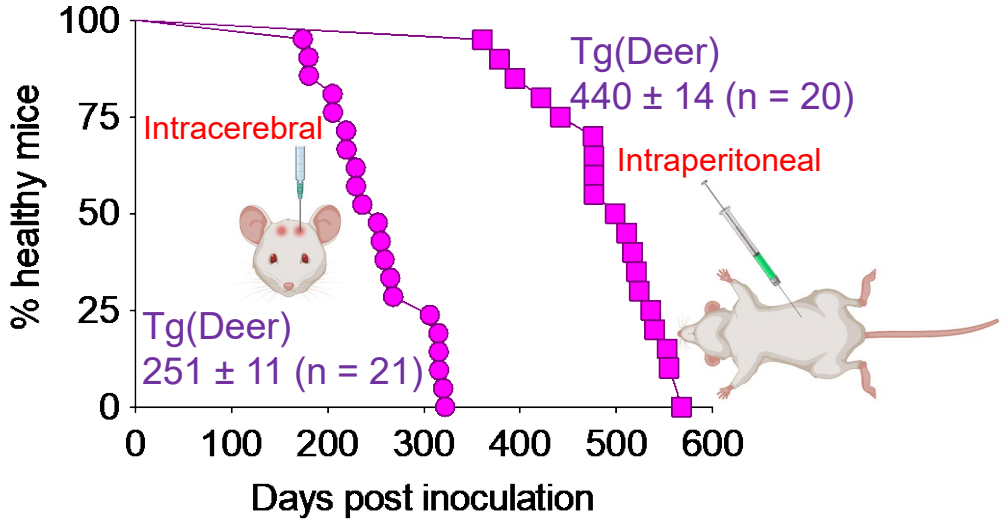


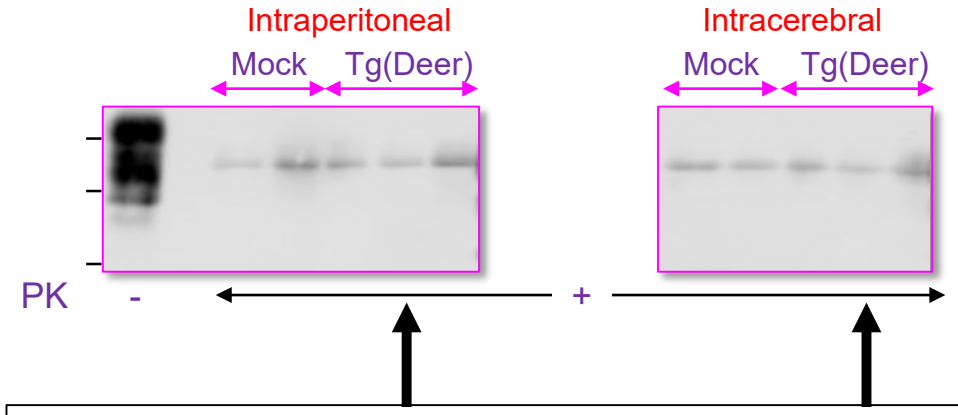
CWD prions are inefficiently transmitted by peripheral routes of exposure in Tg mice

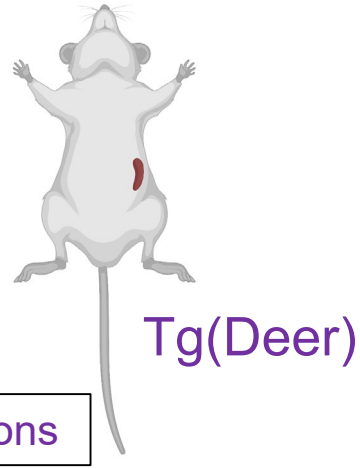


Intraperitoneally-challenged Tg(Deer) mice have significantly longer incubation times than their intracerebrally-challenged counterparts

CWD prions are inefficiently transmitted by peripheral routes of exposure in Tg mice



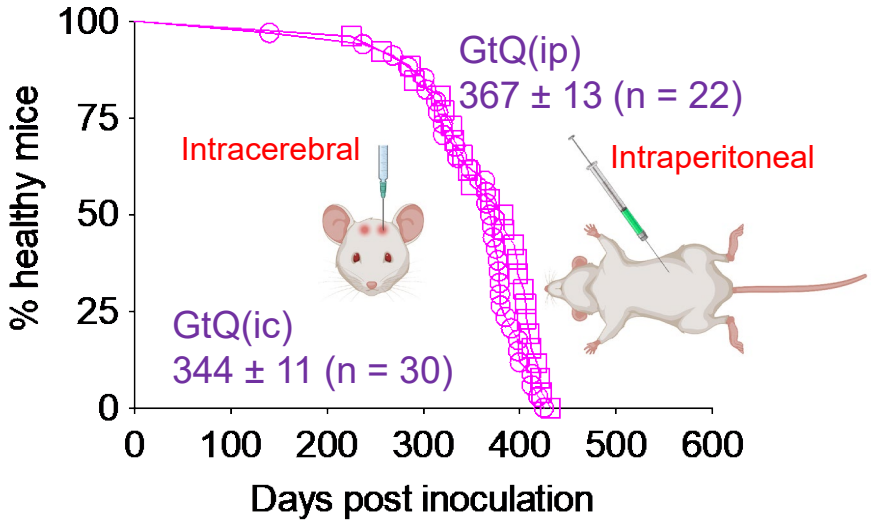




Tg(Deer)

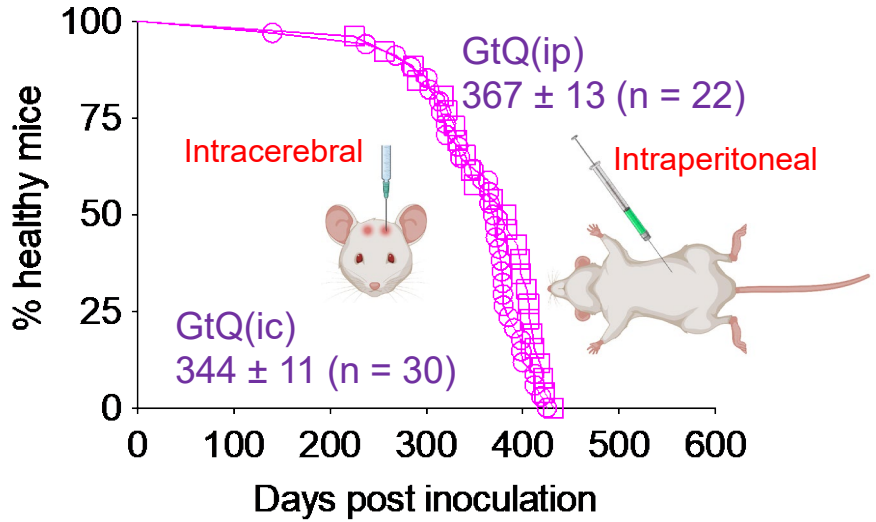
Spleens of diseased Tg mice fail to accumulate CWD prions

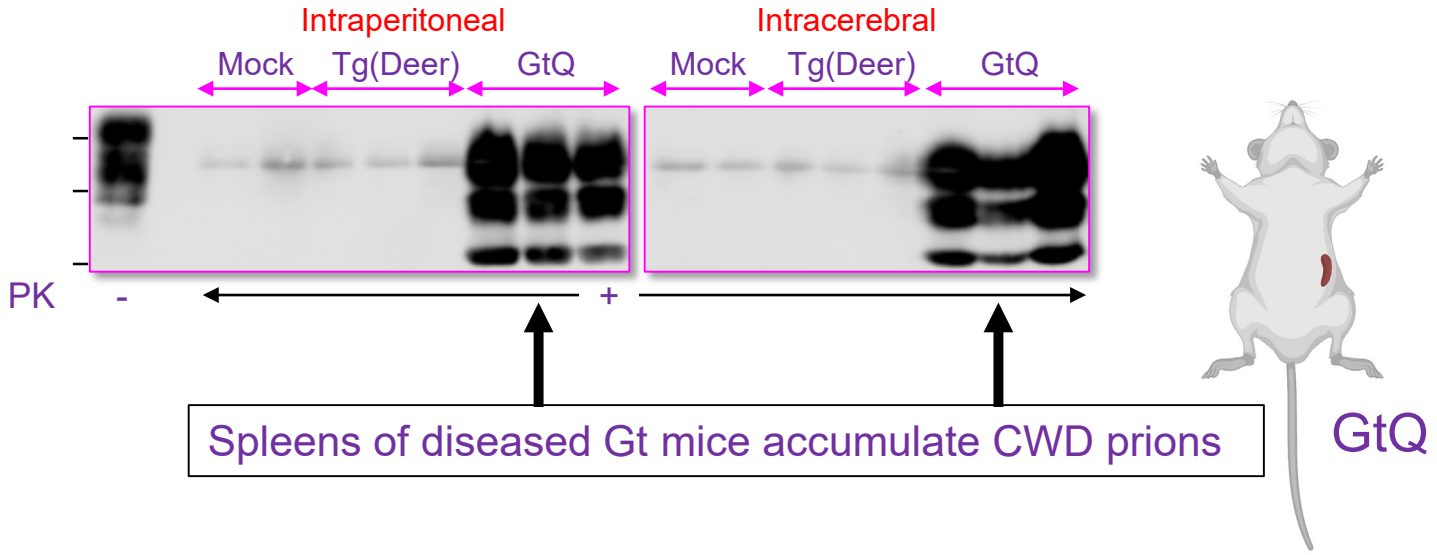
CWD prions are efficiently transmitted by peripheral routes of exposure in Gt mice



By contrast, intracerebrally and intraperitoneally challenged GtQ mice have indistinguishable incubation times

CWD prions are efficiently transmitted by peripheral routes of exposure in Gt mice





Propagation of different strains during peripheral and intracerebral transmissions of CWD to Gt mice

PNAS RESEARCH ARTICLE | BIOCHEMISTRY

Propagation of distinct CWD prion strains during peripheral and intracerebral challenges of gene-targeted mice

Joseph P. DeFranco[a], Jifeng Bian[a,1], Sehun Kim[a], Jenna Crowell[a], Tomás Barrio[a,2], Bailey K. Webster[a], Zoe N. Atkinson[a], and Glenn C. Telling[a,3]

Affiliations are included on p. 10.

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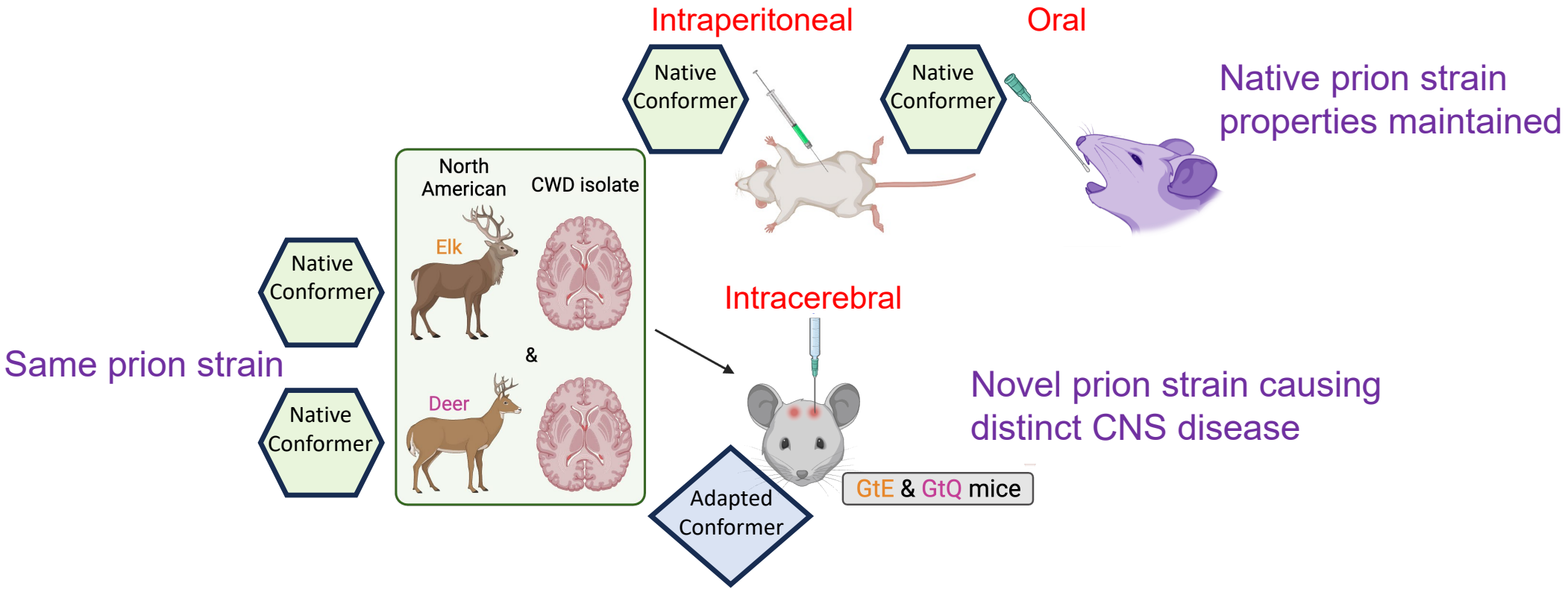


Gene targeted (Gt) mice expressing HuPrP^{C}-M129 and HuPrP^{C}-V129

GtM129

GtV129

- Our results with GtE and GtQ mice indicated that targeted, physiological expression of PrP from *Prnp* provides an improved platform to assess prion strain diversity, and the role of peripheral compartments in strain selection/adaptation

- By extension, these findings also suggest that gene targeted mice offer a refined approach to address the zoonotic potential of prions from diseased animals (e.g. CWD)

- We created Gt mice expressing HuPrP-M129 of HuPrP-V129 from the *Prnp* locus

Transmissions of sCJD prions

GtM129

GtV129

RES-1001 (sCJD brain (MM1)
RES-1002 (sCJD brain (VV1)
RES-1003 (sCJD brain (VV2)
RES-1004 (sCJD brain (MM1)
RES-1005 (sCJD brain (VV2)
RES-1006 (sCJD brain (MM1)

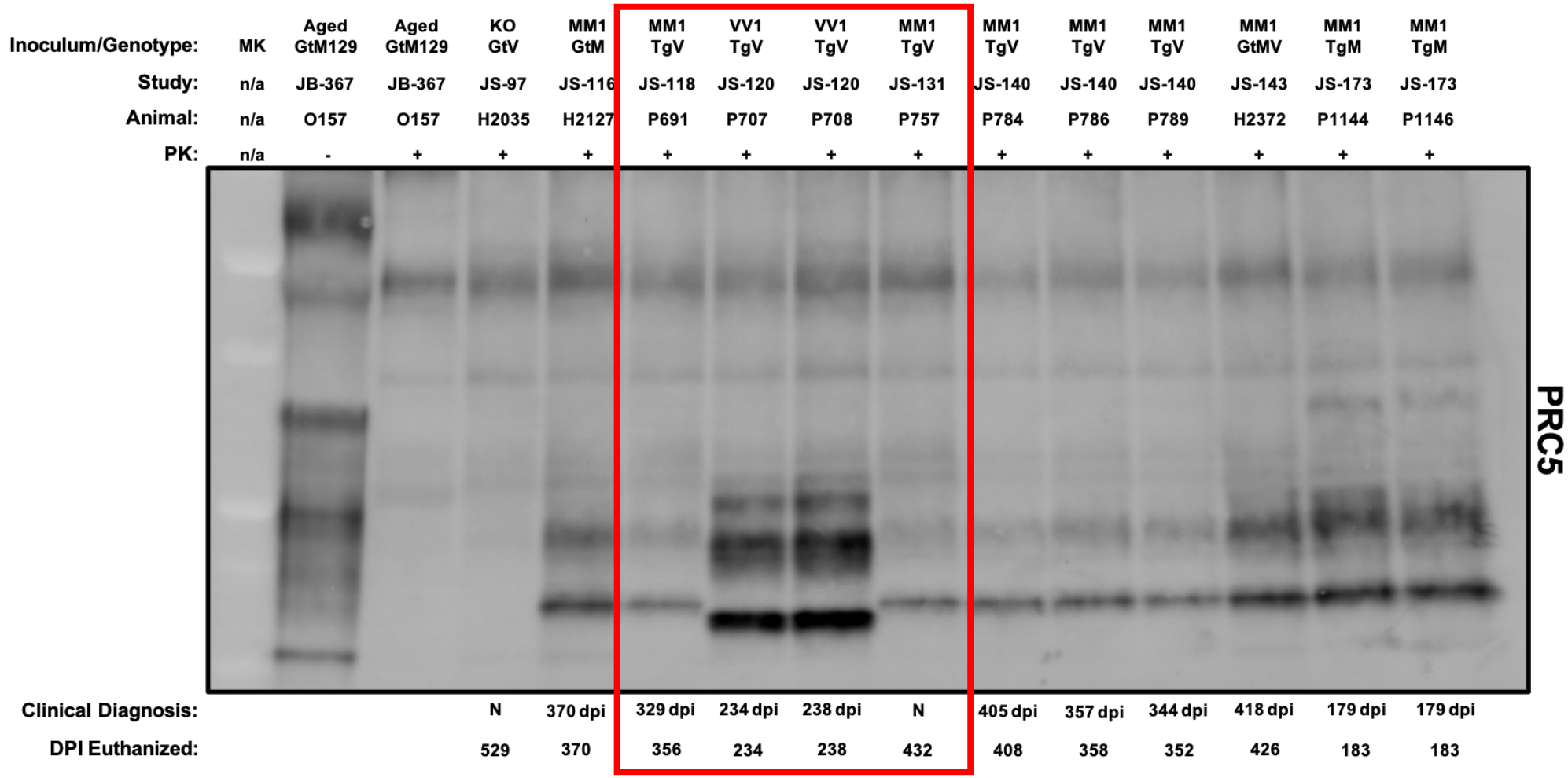
- Dr. Gambetti at Case Western provided six sCJD patient brain samples for transmission studies in Gt mice expressing human PrP
  - Also inoculated Tg human mice
    - TgM ~16x WT
    - TgV ~2x WT
- Mice were either intracerebrally or intraperitoneally inoculated

Intracerebral MM1 sCJD transmissions

RES-1001	RES-1004	RES-1006
GtM/M 297 ± 10 (8/8)	GtM/M 297 ± 6 (4/4)	GtM/M 327 ± 9 (7/7)
GtM/V 440 ± 13 (5/5)	GtM/V 369 ± 7 (7/7)	GtM/V 369 ± 8 (7/7)
GtV/V 532 ± 6 (8/8)	GtV/V 430 ± 26 (7/7)	GtV/V 480 ± 24 (8/8)

Intracerebral sCJD transmissions

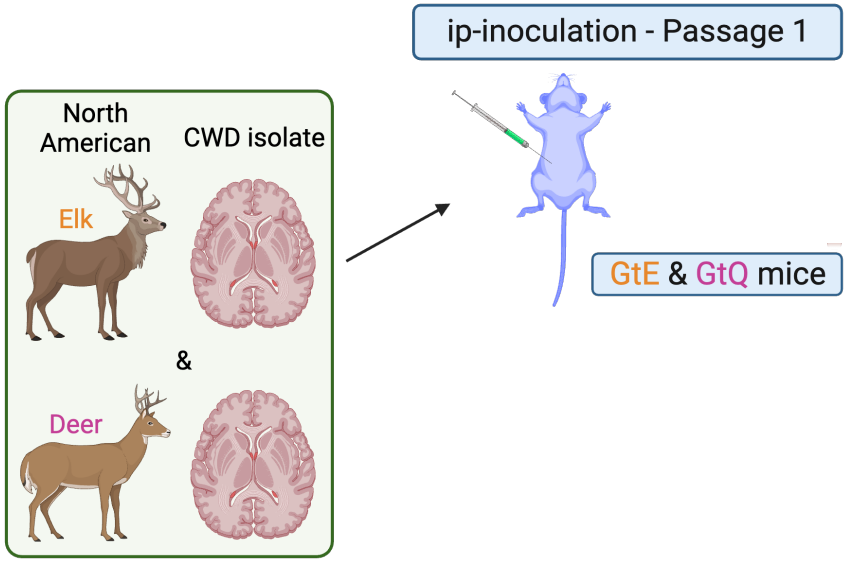
MM1 sCJD	GtM/M	308 ± 13 (19/19)	TgM	175 ± 2 (22/22)
	GtM/V	387 ± 9 (19/19)		
	GtV/V	492 ± 12 (23/23)	TgV	341 ± 12 (22/22)
VV1 sCJD	GtM/M	>566 (0/5)	TgM	516 ± 10 (3/6)
	GtM/V	>554 (0/8)		
	GtV/V	293 ± 4 (6/6)	TgV	231 ± 4 (8/8)
VV2 sCJD	GtM/M	>594 (0/14)	TgM	492 (1/9)
	GtM/V	>553 (0/13)		
	GtV/V	314 ± 4 (11/11)	TgV	242 ± 5 (10/10)

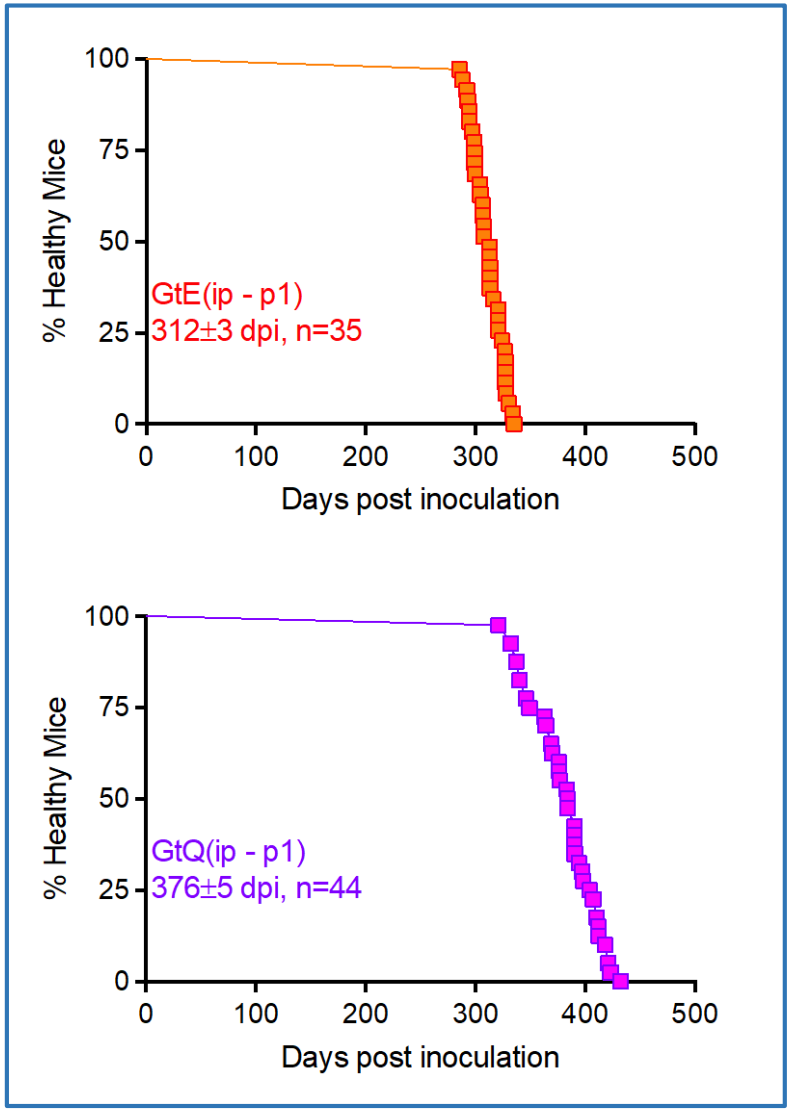


Immunoblot profile[d]

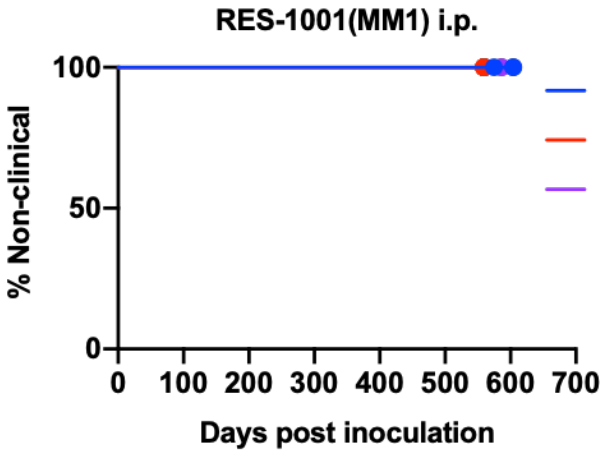
Type 1	Type 2
~21 kDa[e]	~19 kDa[e]

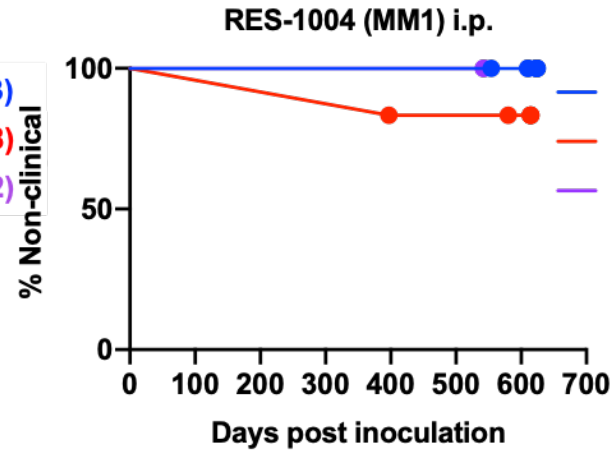
GtE and GtQ succumb to peripheral CWD challenges

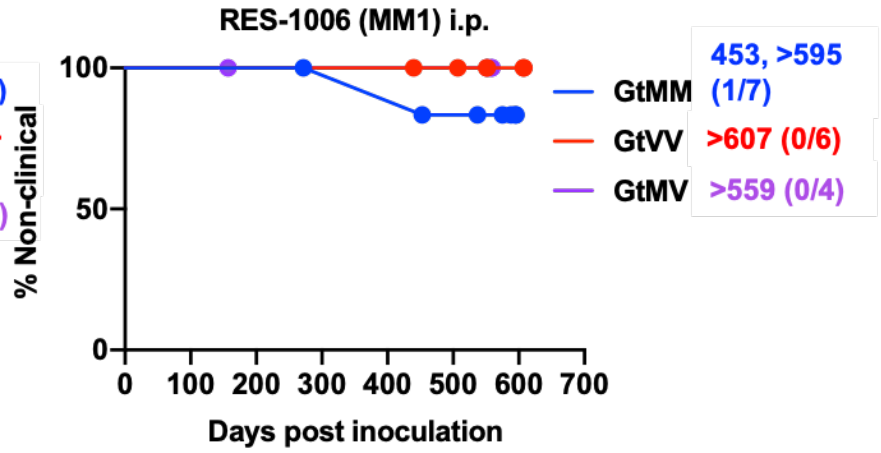


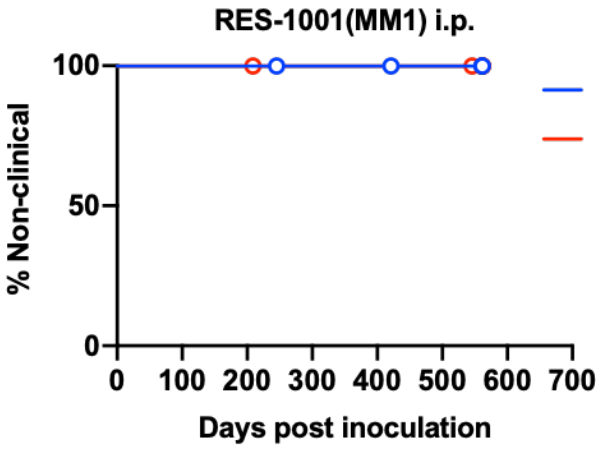


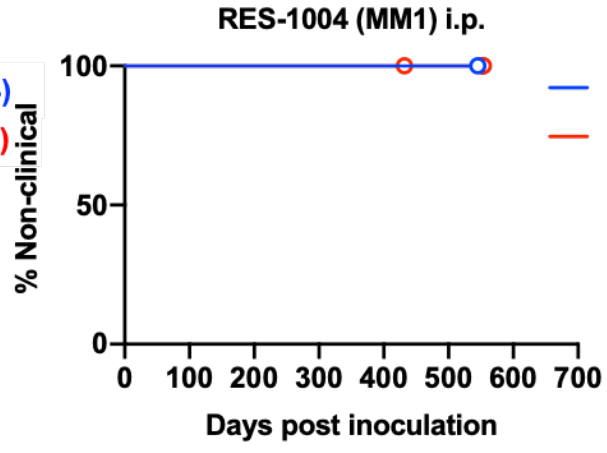
Intraperitoneal MM1 sCJD transmissions

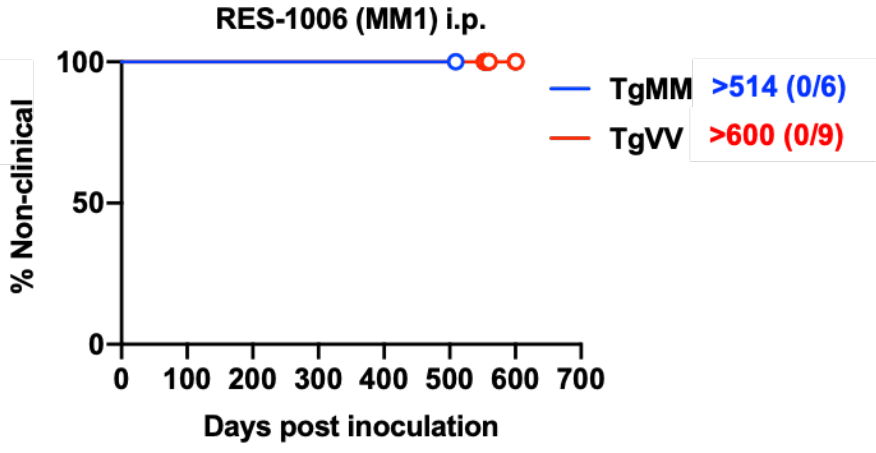




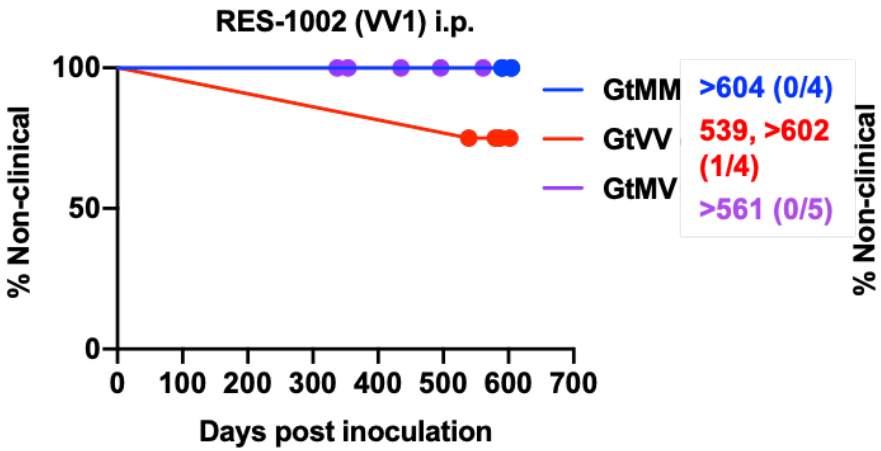


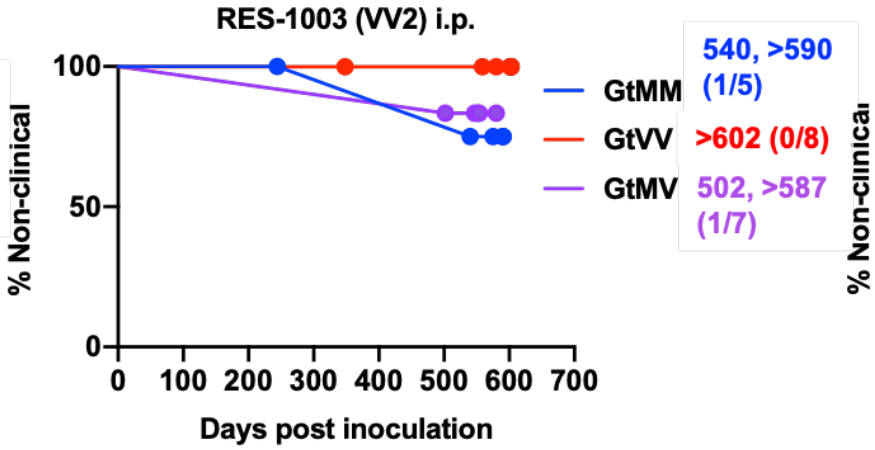


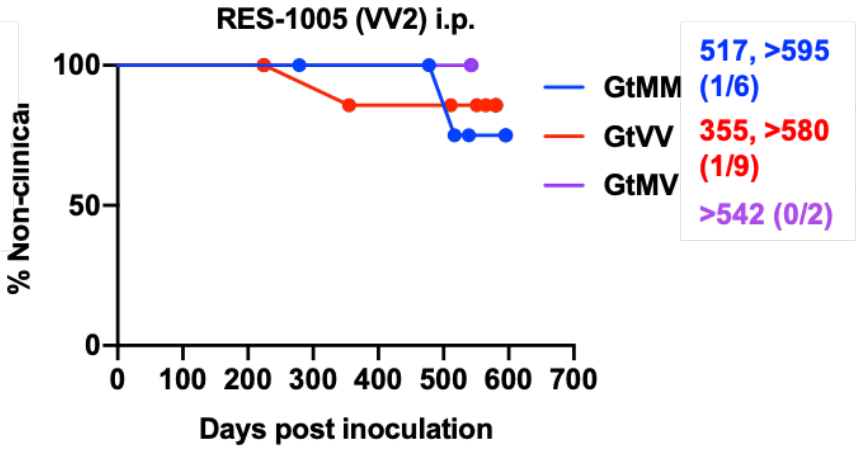


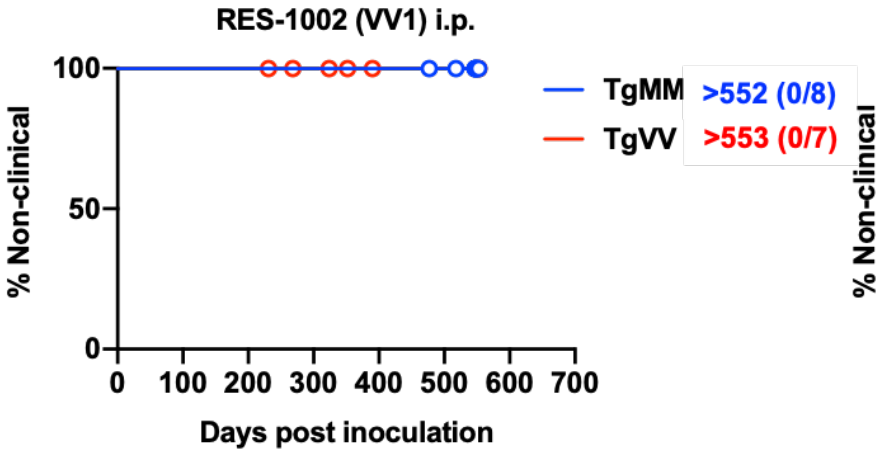


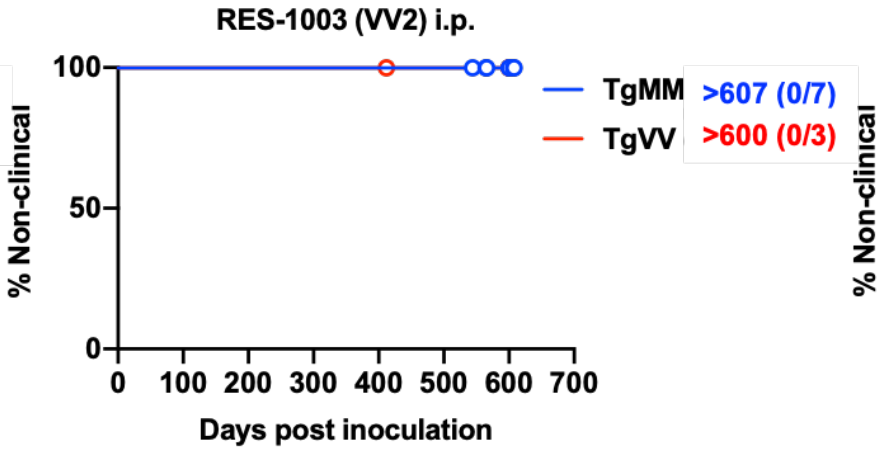
Intraperitoneal VV1 or VV2 sCJD transmissions

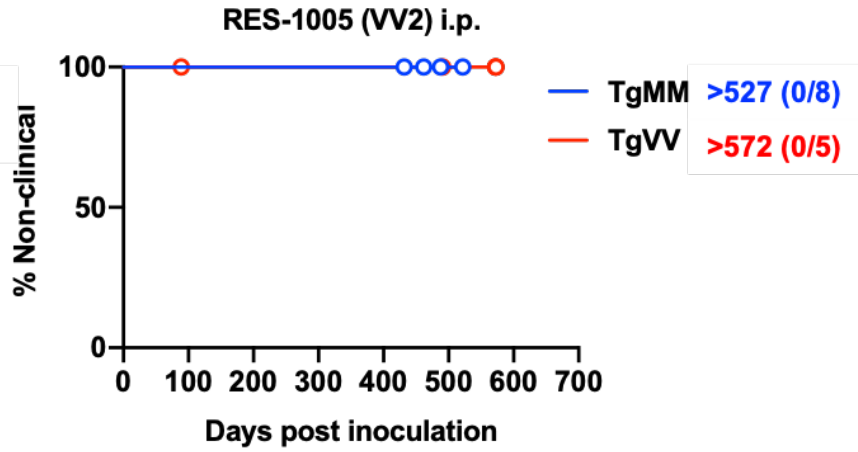












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