Prion 2023

16-20 October

Faro, Portugal

Programme Book

Welcome Message

On behalf of the Prion2023 Congress Organizing Committee and the NeuroPrion Association, we invite you to join us for the International Conference Prion2023 from 16-20 October 2023 in Faro, Portugal.

The Prion2023 Congress in Faro will follow the Prion 2020/22 meeting in Göttingen, the Prion2017 in Edinburgh, Prion2018 in Santiago de Compostela and Prion2019 in Edmonton. We intend to follow the format as well as high standard and quality of the previous meetings. The Prion2023 aims to bring together leading scientists in the field of prion- and prion-like disorders and will discuss the latest developments in structural biology, prion propagation, transmission, animal and human diseases. We will encourage and foster lively discussions about the nature of the agent, the risk to human and animal health, and emerging therapeutic concepts.

We hope to attract the participation of young scientists at early stages in their careers, and will provide ample opportunities for them to present their work. We have allocated considerable space for selected oral communications.

The program will comprise keynote lectures dedicated to specific topics. Oral presentations and poster sessions will be selected from submitted abstracts. Pre-conference workshops and patient organization meetings will be part of the program, and we will include a hot-topics session at the end of the conference.

Faro is a university city on the coast in the southern region of Algarve, providing a fantastic backdrop to the congress. The venue of the conference will be the university lecture hall, where all facilities needed for the conference are available.

We look forward to meeting you in Faro in 2023!

On behalf of the PRION2023 Organizing Committee
Inga Zerr
Herman Schaetzl
Jesus Requena
Jean-Philippe Deslys
Heather True
Amanda Woerman
Leonor Orge
Eduardo Melo
Tuane Vieira
Glenn Telling
Jiyan Ma
Tiago Outeiro

The Algarve: the warm and beautiful southern coast of Portugal

Faro: A City On The Beach

Faro's beaches and the Ria Formosa, where flamingos can be seen taking to the air, mark the border with the sea.

On the flat terrain behind are the houses and buildings of Faro, green vegetable plots that thrive on the fertile land and water wheels that were once used to draw water from the ground another reminder of the city's Moorish heritage.

In the distance, a semi-circle of gentle hills, their slopes clad with fruit trees, frames the landscape. Villages where life goes on at the same easy pace as in centuries past, where unassuming churches conceal art works of astonishing beauty, where vestiges of the magnificence of the Romans still litter the fields.

These are among the charms of Faro and its municipality, an ideal starting point for exciting voyages of discovery.

Olhão

The islands and the long stretches of beach that are an ideal spot for swimming and sunbathing.

The tranquil waters of the Ria Formosa, a paradise for nature lovers; and in the background, the countryside dotted with white houses: these are the attractions of Olhão and its municipality, a great place for a holiday full of sun, life and a whole host of charms.

Lagos

Gigantic sculptures carved by the pounding waves that plunge into a crystal sea.

The iridescent greens of sea caves. Beach after beach of soft sand tucked away between ochre cliffs or stretching clear to the horizon. Verdant countryside dotted with the white of houses.

Just a few reasons for visiting and discovering one of the most attractive parts of the Algarve.

Sagres

The mythical atmosphere surrounding Sagres and Cabo de São Vicente (Cape St. Vincent), places dedicated to the gods for thousands of years.

The unspoilt coastline, with its dramatic horizons of cliffs and sea; the many menhirs that bear witness to prehistoric rites; memories of the epic of the Discoveries and the enigmatic figure of Prince Henry the Navigator: such are the attractions of Vila do Bispo

and its municipality, a vast triangle where the sea is a constant presence. And where nature combines with history to create a unique region that is well worth getting to know.

Lagoa

A turquoise sea bounded by ochre cliffs and soft, sandy beaches.

Bunches of grapes ripening beneath the hot summer sun. The shapes, colors and designs of ceramics that belong to a tradition centuries old.

Such are the attractions of Lagoa and its surroundings, the romantic chapel of Nossa Senhora da Rocha perched high above the sea, and the fascinating rock formations of Algar Seco.

Albufeira

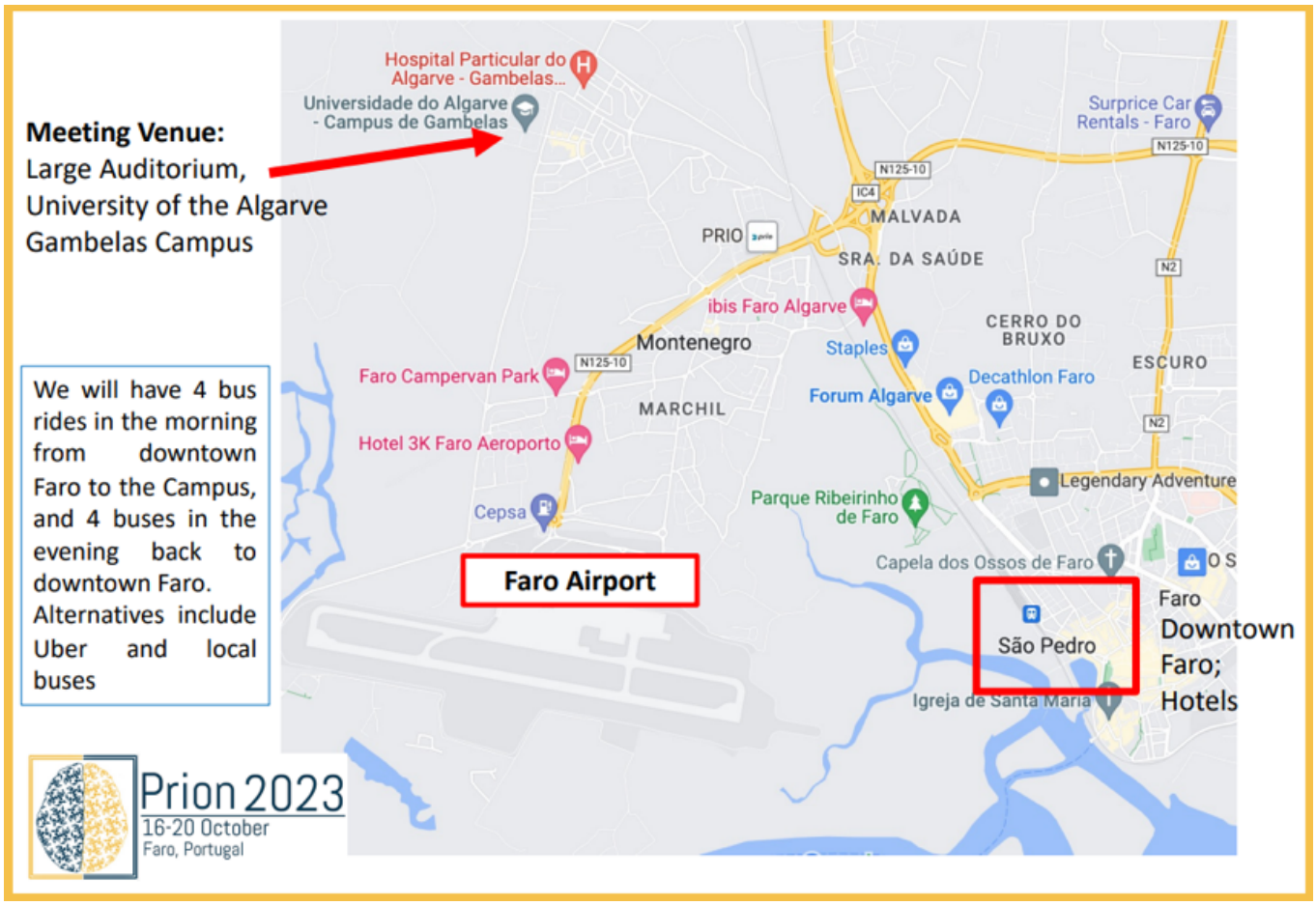
First and foremost, Albufeira is famed for its beaches, for the countless shades and tones of its rocks and cliffs.

This is a place where people live to the rhythm of the great holiday destinations, sunbathing during the day and at sunset flocking to enjoy the restaurants, bars and discotheques that enliven the nighttime hours.

Just a few miles inland and everything changes. The green of the countryside is dotted with almond, fig, orange and pine trees and decorated chimneys stand out against the ochre of tiled roofs. Bucolic villages invite you to experience a way of life rooted in the tranquility of nature, to add another dime

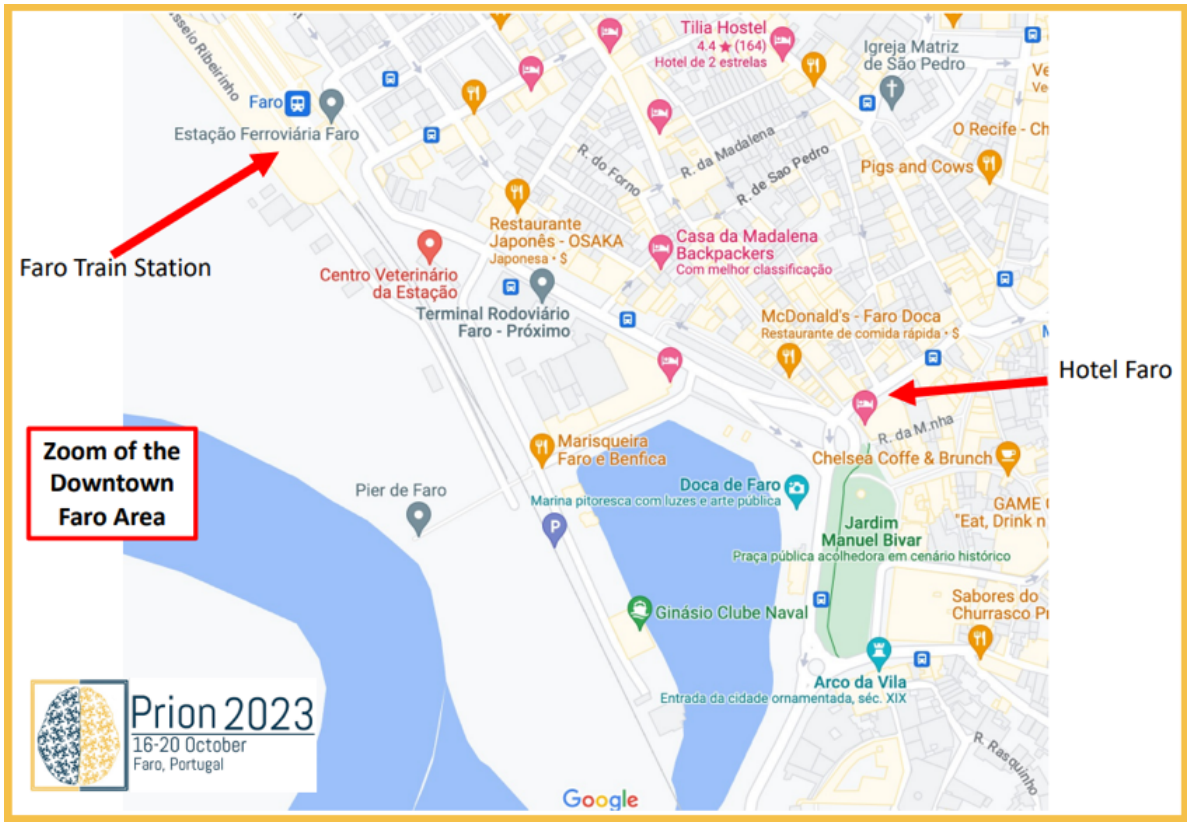
Meeting Venue

Large Auditorium
Gambelas Campus
University of the Algarve
Faro, Portugal



Downtown Faro

Most hotels are located in downtown Faro, situated about 15 minutes away from the Gambelas Campus of the University of the Algarve, where the meeting will take place.



You can access the University by taxi, Uber, local buses, or using the buses organized by the meeting. Bus pick up and drop off in downtown Faro is shown below. Buses will take you directly to the University. No additional stops will be allowed, so ensure you are at the pick up location on time.

Bus schedule

Downtown Faro to University (Gambelas Campus)

Day	Pick up downtown Faro	Arrival at University	Notes (no additional stops possible)
October 16	13h	13h20	One pick up only
October 17	07h45 / 08h30	08h05 / 08h50	Two pick ups only
October 18	07h45 / 08h30	08h10 / 08h50	Two pick ups only
October 19	07h45 / 08h30	08h10 / 08h50	Two pick ups only

University (Gambelas Campus) to Downtown Faro

Day	Pick up University	Arrival in downtown Faro	Notes (no additional stops possible)
October 16	18h30	18h20	One pick up only
October 17	20h00 / 20h45	20h20 / 21h05	Two pick ups only
October 18	19h30 / 20h15	19h50 / 20h35	Two pick ups only
October 19	22h15 / 22h45	22h35 / 23h05	Two pick ups only

General Information

Registration Desk
The registration desk will open on October 16 at 13h00, and will be open from 8h00 to 18h00 on October 17-19.

Name Badge Policy
Name badges should be worn at all times to identify the registered participants.

Meeting Programme and abstract book
To reduce paper usage, the meeting programme can be found below, and online at prion2023.org.

Poster sessions
Poster sessions will take place during all breaks in order to enable maximal interaction between participants. Please ensure you stand by your poster as often as possible.

Certificate of Attendance
Certificates of attendance will be emailed after the meeting.

Meals
Coffee breaks will be served outside the auditorium.
Registered participants will receive tickets for lunches and for the Gala dinner.

Recording and photography
Recording and photography of lectures and posters is forbidden.

Mobile phones
We kindly ask you to mute your mobile devices during the lectures.

Smoking
Smoking/vaping inside the University buildings is strictly forbidden.

Emergencies
For any fire, police, or health emergencies in Portugal, please dial 112.

Workshops

Venue

Large Auditorium
University of the Algarve, Gambelas Campus
Faro, Portugal

Monday, October 16

Joint Introduction

14:00h – 14:30h	History of animal prion diseases	Jason Bartz
14:30h – 15:00h	History of human prion diseases	Richard Knight

15h00 to 18h00 Parallel workshops – **The parallel workshops will take place in three separate rooms. Participants will be guided to the rooms by local staff.**

W1. Neuropathology and clinicopathological correlation of human prion diseases and related dementias – Organized by Markus Glatzel

Several dementias are characterized by aggregation of abnormally folded conformers of host-encoded proteins. In prion diseases and related dementias, seeded aggregation can lead to the spread of protein aggregates throughout the brain.
In this workshop, we will focus on neuropathology, selective cellular and regional vulnerability, and clinicopathological correlation not only in Creutzfeldt-Jakob disease but also in Parkinson's, Alzheimer's disease and related dementias. Besides lectures by prominent experts in the field such as Zane Jaunmuktane, Gabor Kovacs and Markus Glatzel there will be room for case discussions and hands-on neuropathology training in this exciting field of science.

- 15:00h – 15:05h: **Markus Glatzel:** Introduction to the concept of this workshop
- 15:05h – 15:20h: **Markus Glatzel:** Pathological deposition of the prion protein and Creutzfeldt-Jakob disease.
- 15:20h – 15:40h: **Jane Jaunmuktane:** Pathological deposition of the A-beta, tau and alpha-synuclein in Alzheimer's and Parkinson's disease
- 15:40h – 16:00h: **Gabor Kovacs:** Sequential distribution patterns of alpha-synuclein and tau in other neurodegenerative diseases.
- 16:00h – 16:30h: **Break**
- 16:30h – 17:45h: All speakers and the audience: Case presentation and discussion with selected cases of CJD, AD, DLB, PSP and MSA.
- 17:45h – 18:00h: **Markus Glatzel:** Summary and feedback.

W2. Cell and animal models of prion and prion-like diseases – Organized by Tiago Outeiro

Several dementias are characterized by the accumulation of abnormally folded conformers of host-encoded proteins. However, the precise molecular underpinnings of neurodegeneration are still unclear. In this context, modeling key disease mechanisms in laboratory models is essential in order to enable the scientific community to test therapeutic strategies.
In this workshop, we will focus on cell and animal models of prion and prion-like diseases. We will have lectures by key experts in the field and time for discussions.

- 15:00h – 15:05h: **Tiago Outeiro:** Introduction of the workshop.
- 15:05h – 15:35h: **Jiayi Li:** Cell models for studying prion-like diseases.
- 15:35h – 16:05h: **Amanda Woerman:** Bridging to the clinic: Mouse models of tau and alpha-synuclein prions
- 16:05h – 16:40h: **Walker Jackson:** Tools to reveal molecular mechanisms in presymptomatic mouse models of Huntington's and prion diseases.
- 16:40h – 17:00h: **Break**
- 17:00h – 17:45h: All speakers and the audience: Discussion.
- 17:45h – 18:00h: **Tiago Outeiro:** Summary and feedback.

W3. Structural biology techniques for studying prion protein in vitro and in silico – Organized by Tuane Vieira

Prion diseases have long been a subject of scientific intrigue and scrutiny due to their multifaceted and elusive nature. A deep understanding of prion protein structures is imperative to comprehend the complex mechanisms at play. In this workshop, we will explore the cutting-edge techniques employed in vitro and in silico to decipher the intricacies of prion proteins.

Our panel of experts will provide profound insights into the latest advancements, methodologies, and discoveries in the field of structural biology, with a focus on their direct relevance to prion diseases.

- 15:00h – 15:05h: **Tuane Vieira:** Introduction of the workshop
- 15:05h – 15:35h: **Yraima Cordeiro:** Spectroscopic techniques to study prion protein misfolding and aggregation.
- 15:35h – 16:05h: **Holger Wille:** Exploring Prion Protein Structure through High-Resolution Techniques
- 16:05h – 16:40h: **Salvador Ventura:** Fifteen years developing computational tools to study protein aggregation
- 16:40h – 17:00h: **Break**
- 17:00h – 17:45h: All speakers and the audience: Discussion
- 17:45h – 18:00h: **Tuane Vieira:** Summary and feedback.

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Meeting Programme

October 17

09:00 Opening Session

Rector of University of the Algarve
Vice-President of Faro City Hall, Dr. Paulo Santos
Dean of the Medical School
President of ABC
Director of ABC-RI
Organizing committee representatives

Session 1. Protein structure, function, conversion, dysfunction

Moderator: Alejandro Ruiz

09:30 – 09:30 Keynote lecture: Szymon Manka, Structural biology of prions in 4D: from *ex vivo*, via *in cellulo,* towards *in situ*

10:00 – 10:15 Runchuan Yan, Flexible N-Terminal Domain of the Prion Protein is Neurotoxic
10:15 – 10:30 Luigi Russo, Structural and mechanistic insights into the pathogenesis of prion diseases
10:30 – 10:45 Ilaria Vanni, Isolation of oligomeric prions from atypical scrapie

10:45 – 11:15 Coffee break + posters

Session 2. From yeast prions to mammalian functional aggregates

Moderator: Heather True

11:15 – 12:00 Keynote lecture: Reed Wickner, Anti-prion systems cull all but one in 5000 prions that arise

12:15 – 12:30 Tayyaba Saleem, Isolation and characterization of stress granules from human brain
12:30 – 12:45 Marta Badia, The mutational landscape of the Sup35 QN-rich domain reveals an essential region for Sup35 nucleation
12:45 – 13:00 Yury Chernoff, Relationship between hyperosmotic stress, liquid-liquid phase separation and prion formation

13:00 – 14:30 Lunch break + posters

Session 3. Spreading of pathology in prion-like diseases
Moderator: Amanda Woerman
14:30 – 15:00 Keynote lecture: Mathias Jucker, A-beta seeds
15:00 – 15: 15 Ina Vorberg, Propagation of yeast prions in a mammalian host
15:15 – 15:30 Shelley Forrest, *MAPT* and *SNCA* gene expression in cells with protein cytopathologies in Tau- and Synuclein- related neurodegenerative diseases
15:30 – 15:45 Farjana Parvin, Recombinant Aβ amyloid fibrils seed CAA pathology in APP23 transgenic mice

15:45 – 16:45 Coffee break + posters

Session 4. Pathogenic mechanisms in prion diseases
Moderator: Eduardo Melo
16:45 – 17:15 Keynote lecture: David Westaway, Beta-endoproteolysis of the cellular prion protein by dipeptidyl peptidase-4 and fibroblast activation protein

17:15 - 17:30 Giuseppe Legname, A novel prion clearance mechanism by SERPINA3/SerpinA3n
17:30 – 17:45 Prerna Grover, The role of the soluble N-terminal domain (N1-PrP) of the prion protein in αSyn Aggregation and Seeding
17:45 – 18:00 Diane Ritchie, Investigating infectivity associated with prion protein deposits detected in formalin-fixed paraffin- embedded specimens from asymptomatic carriers (UK Appendix studies): an initial update

18:30 Art of Science Event and Welcome Reception

October 18

Session 5. The connection between prion diseases and other disorders
Moderator: Tuane Vieira
09:00 – 09:30 Keynote lecture 1: Jerson Silva, Prion-like Aggregation and Phase Transition of Mutant p53 as a Novel Cancer Treatment Strategy

09:30 – 10:00 Keynote lecture 2: Sophie Mouillet-Richard, The prion protein from neurodegeneration to cancer

10:00 – 10:15 Elizabeth Hill, Assessing the Role of Syntaxin-6 on Prion Initiation, Prion Propagation and Prion-Induced Neurotoxicity *in vivo*
10:15 – 10:30 Holger Wille, A unified approach for rationally designed vaccines targeting neurodegenerative diseases
10:30 – 10:45 Carmen Nussbaum-Krammer, Cellular disaggregation and degradation systems generate seeding and spreading-competent α-Syn and Tau species

10:45 – 11:15 Coffee break + posters

Session 6. Cell biology of PrP

Moderator: Jiyan Ma

11:15 – 11:45 Keynote lecture 1: Christina Sigurdson, Deranged synaptic signaling in experimental prion disease

11:45 – 12:15 Keynote lecture 2: Chiara Zurzolo, Mechanisms of spreading of prions: role of tunneling nanotubes

12:15 – 12:30 Nadia A. Mirza, Murine astrocytes display differential susceptibility to prion strains

12:30 – 12:45 Patricia Aguilar-Calvo, Reducing the sulfation of neuron-derived heparan sulfate accelerates prion protein clearance and prolongs survival time in prion-infected mice

12:45 – 13:00 Walker Jackson, Expression of Rps24-PKE is induced by activated microglia during acquired and genetic prion diseases

13:00 – 14:30 Lunch break + posters

Session 7. Animal prion diseases

Moderator: Leonor Orge

14:30 – 15:00 Keynote lecture: Glenn Telling, Refined transgenetic approaches to characterize the host-range and adaptive potentials of emerging and established chronic wasting disease strains

15:00 – 15:15 Audrey Sandoval, Chronic wasting disease *in utero* transmission in free-ranging white-tailed deer

15:15 – 15:30 Sabine Gilch, Norwegian moose CWD induces clinical disease and spleen-independent neuroinvasion in mice expressing cervid S138N prion protein

15:30 – 15:45 Francisca Bravo Risi, Detection of CWD prions in plants collected from white-tailed deer farms

15:45 – 16:30 Coffee break + posters

Session 8. Transmission of prions and prion-like proteins

Moderator: Tiago Outeiro

16:30 – 17:00 Keynote lecture: Jiyan Ma, Understanding the seeding capability, transmissibility, and pathogenicity of prion

17:00 – 17:15 Sarah A. M. Holec, Effect of neuroinvasion on strain property maintenance for two a-synuclein prion strains Hornberg, Propagation of yeast prions in a mammalian host

17:15 – 17:30 Joel Watts, Stochastic misfolding drives the emergence of distinct a-synuclein strains

17:30 – 17:45 Rodrigo Morales, Deciphering the pathological significance of self-propagating Aβ strains in different animal models

18:00 – 19:00 Special lecture – John Collinge, A brief history of prions

October 19

Session 9. Biomarkers for prion and neurodegenerative diseases
Moderator: Matthias Schmitz
09:00 – 09:30 Keynote lecture: Inga Zerr, Biomarkers for prion and neurodegenerative diseases

09:30 – 09:45 Wen-Quan Zou, Large-Scale Validation of Skin Prion Seed-Amplification Assay for Diagnosis of Creutzfeldt- Jakob Disease
09:45 – 10:00 Michael Geschwind, General and biomarker cerebrospinal fluid findings in prion disease and other rapidly progressive dementias
10:00 – 10:15 Sang-Hyun Oh, Blood-based nano-QuIC: Accelerated and Inhibitor-resistant Detection of Misfolded a-synuclein

10:15 – 11:00 Coffee break + posters

Session 10. CJD International Support Alliance
11:00 – 12:30 Supporting patient associations globally and providing a unified, global voice for all affected by prion disease

12:30 – 14:00 Lunch break + posters

Session 11. Current therapeutic approaches in neurodegeneration
Moderator: Inga Zerr
14:00 – 14:30 Keynote lecture: Jeffrey Kordower, Current therapeutic approaches in neurodegeneration

14:30 – 15:00 Sonia Vallabh, Broad Institute, Lowering PrP: Evidence of therapeutic benefit across diverse yet relevant models
15:00 – 15:30 Pekka Kallunki, Lundbeck, Inhibition of prion like seeding of pathology in Parkinson's disease by antibodies and other therapies to alpha-synuclein in clinical development
15:30 – 16:00 Warren Hirst, Biogen, Therapeutic approaches for AD

16:00 – 16:45 Coffee break + posters

Session 12. Late breaking news
Moderator: Glenn Telling
16:45 – 17:00 Joaquín Castilla, A Noah's ark approach to understand the molecular basis of the bona fide spontaneous prion misfolding
17:00 – 17:15 Marcela Viviana Karpuj, A Novel Therapeutic Approach Modulates Protein Interactions and Cellular Processes
17:15 – 17:30 Vineet Rathod, Engineering of a single chained fluobody into a flashbody format for optimized detection and labeling of native PrPSc

17:30 – 17:45 Joseph DeFranco, Peripherally-challenged gene-targeted mice produce strains that recapitulate the properties of natural CWD and are distinct from intracerebrally-adapted variants

18:00 Closing session

19:00 Gala dinner

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Submitted Abstracts

Abstract List

Invited lectures

A Szymon Manka
B Reed Wickner
C Mathias Jucker
D David Westaway
E Jerson Silva
F Sophie Mouillet-Richard
G Christina Sigurdson
H Chiara Zurzolo
I Glenn Telling
J Jiyan Ma
K Inga Zerr
L Jeffrey Kordower
M Sonja Vallabh
N Pekka Kallunki
O Warren Hirst

General submissions (in alphabetical order of first name)

(Numbers correspond to poster board numbers where posters should be hanged)

1 Abrar Younas
2 Alberto Bizzi
3 Alexia Frese
4 Alicia Otero
5 Alyssa Block
6 Amy Nalls
7 Anna Burato
8 Anthony Kincaid
9 Arianna Ciullini
10 Arielle Hay
11 Audrey Sandoval
12 Avery Lessard
13 Barbara Altenhuber
14 Belinda Ameyaw
15 Brenda Rajanyagam
16 Caitlyn Kraft
17 Carmen Garcia Pelayo
18 Carmen Nussbaum
19 Cathryn Haigh
20 Daniel Shoup
21 Daniela Meloni/Giuseppe Ru
22 Debora Foguel
23 Diana Lowe
24 Diana Ritchie
25 Dieter Willbold
26 Dimitra Dafou 1

27 Dimitra Dafou 2
28 Dominika Ewa Serwin
29 Duncan Brown / Steve Kymes
30 Elaine Petronilho
31 Elisabeth Hill
32 Emiliano Biasini
33 Emanuel Comoy
34 Enric Vidal Barba
35 Eric Cassmann
36 Erika Bronzato
37 Erin McNulty
38 Fabricio Cruz-Lopez
39 Farjana Darvin
40 Frederico Angelo Cazzaniga
41 Fernando Morais
42 Francisca Bravo Risi
43 Friederike Schaper 1
44 Friederike Schaper 2
45 Gerold Schmitt-Ulms
46 Giuseppe Bufano
47 Giuseppe Legname 1
48 Giuseppe Legname 2
49 Hien Zhao
50 Holger Wille
51 Ilaria Vanni
52 Ina Vorberg
53 Inigo Chomon
54 Irina Derkatch
55 Ivan Martinez-Valbuena
56 James Carroll
57 James Striebel
58 Jan Bieschke
59 Jan Mackenzie
60 Janet Hills
61 Jennifer Hoy Petersen
62 Jessica Cashion
63 Jesus Requena
64 Joaquin Castilla Castrillon
65 Joel Watts
66 Johan Larsson
67 Joost Schymkowitz
68 Joseph Abrams
69 Joseph DeFranco
70 Juan Carlos Espinosa
71 Justin Greenlee
72 Kaitlyn Forest
73 Kalpshree Gogte
74 Katie Williams
75 Kylee Drever
76 Lauren Pourghaderi
77 Leonardo Cortez
78 Liyong Wu 1
79 Liyong Wu 2
80 Lovney Kanguru
81 Luigi Russo
82 Malin Sandberg
83 Marcela Viviana Karpuj
84 Maria Leonor Orge
85 Maria Rebeca Benavente
86 Mariam Ansari
87 Marta Badia Graset
88 Matilde Bongianni
89 Matthias Schmitz
90 Melissa Rayner
91 Michael Geschwind 1
92 Michael Geschwind 2
93 Min Chu
94 Mourad Tayebi
95 Nadia Mirza
96 Natalia Gorsheneva
97 Natalia Ventserova
98 Nathaniel Denkers
99 Neelam Younas 1
100 Neelam Younas 2
101 Niccolo Candelise
102 Noa Bregman
103 Nurit Omer
104 Otto Windl
105 Patricia Aguillar-Calvo
106 Paulina Soto
107 Peter Larsen
108 Prerna Grover
109 Qingzhong Kong 1
110 Qingzhong Kong 2
111 Rachel Shoemaker
112 Ricardo Pascuzzo
113 Robert Mercer
114 Rodrigo Morales 1
115 Rodrigo Morales 2
116 Romolo Nonno
117 Ronald Shikiya
118 Runchuan Yan

119 Ruth Gabizon
120 Ryan Sayers
121 Sabine Gilch
122 Sam Koshy
123 Sang-Gyun Kang
124 Sang-Hyun Oh 1
125 Sang-Hyun Oh 2
126 Sara Canoyra Sanchez
127 Sara Holec
128 Sara Zurbuchen
129 Sebastian Mignacca
130 Szegi Canaslan Eyyuboglu
131 Shelley Forest
132 Shi-Wie Chou
133 Sofie Nyström
134 Sona Baranova
135 Sonja Ernst
136 Stuart Lichtenberg
137 Sue-Ann Mok
138 Surabhi Mehra
139 Suzanne Suleiman
140 Tadashi Tsukamoto
141 Tahir Ali 1
142 Tahir Ali 2
143 Takashi Hoshika
144 Takehiro Nakagaki
145 Taylor Corridon
146 Tayyaba Saleem
147 Temuulen Erdenebat
148 Thomas Trainer
149 Tiago Outeiro
150 Tiffany Wolf
151 Timm Konold
152 Tuane Vieira
153 Vanessa Laversenne
154 Vangelis Bouris
155 Vineet Rathod
156 Walker Jackson
157 Waqas Tahir
158 Wenquan Zou 1
159 Wenquan Zou 2
160 Yraima Cordeiro
161 Yuri Chernoff
162 Zoe Lambert

Structural biology of prions in 4D: from *ex vivo*, via *in cellulo,* towards *in situ*

Szymon W. Manka

Institute of Prion Diseases and MRC Prion Unit at UCL, University College London, London, UK

Three-dimensional cryogenic electron microscopy (cryo-EM) of various *ex vivo* rodent prion strains has demystified the general way in which prion protein (PrP) monomers assemble into overtly infectious prion fibrils in the brain. The common two-lobed prion fibril architecture contains variable motifs, serving as unique, strain-differentiating features. Over the last year, we have focused on the fourth dimension, pertaining to prion infection dynamics. We set out to address the remaining knowledge gaps using prion-susceptible cell cultures and cryogenic correlative light and electron microscopy (cryo-CLEM). Success with this work will lay the foundation for *in situ* electron cryo-tomography, to detect all disease-relevant prion assembly states directly in infected brain tissue.

B

Anti-prion systems cull all but one in 5000 prions that arise.

Reed B. Wickner, Herman K. Edkses, Moonil Son*, Songsong Wu and Kristen Gregg

Laboratory of Biochemistry and Genetics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0830 USA

*Present address: Busan National University, Busan, South Korea

Aims: Curing prion diseases.

Methods: Yeast molecular genetics.

Results and Conclusions: The [PSI+] and [URE3] prions are diseases of yeast based on the prevalence of toxic 'prion strains', and the rarity of even mild prions in wild strains. Yeast systems (in normal cells) block prion generation, propagation, segregation to daughter cells and the toxic effects of these prions. Btn2 sequesters prion and other aggregates, Upf1,2,3 directly interact with Sup35 blocking amyloid formation, Hsp104 lowers prion generation >10-fold and cures some prion strains, ribosome-associated chaperones prevent misfolding of nascent proteins and Siw14 is a pyrophosphatase degrading certain inositol polyphosphates important for [PSI+] propagation. We find that [PSI+] appears up to 5000-fold more often in cells defective in three of these systems (*upf1Δ ssz1Δ hsp104*^{T160M}) than in wild type. Most of the prions arising in the triple mutant are cured by replacement of any one of the defective genes, showing that these three systems work independently. Such prions represent new strain types not previously detected in studies of the single mutants (or other yeast strains). We show that generation of prions stable in wild type cells is also blocked 25 to 100 fold by these three systems. Human prions are now known to have the same folded in-register parallel architecture as we described in yeast prions, so it is hoped that human anti-prion systems analogous or homologous to those we have found in yeast can be found and used in treatment of prion and amyloid diseases. Accordingly, we have identified 19 human proteins whose expression in yeast cures yeast prions.

Funded by: the Intramural Program of the National Institute of Diabetes and Digestive and Kidney Diseases of the U.S. National Institutes of Health, grant number: ZIA DK024950-17

Theme: Yeast Prions.

Aβ seeds
Mathias Jucker

The commonality of many neurodegenerative disorders is the progressive temporal and spatial aggregation of specific proteins in the brain. The quintessential proteopathy is Alzheimer's disease (AD), in which the aggregation and seeded propagation of amyloid-β peptide (Aβ) triggers AD pathogenesis, including neuronal Tau inclusions and neurodegeneration. Current therapeutic strategies focus on early disease stages and aim to inactivate Aβ seed propagation before the onset of neurodegeneration. However, to develop such primary prevention approaches a mechanistic understanding of early disease stages is essential.

D

Beta-endoproteolysis of the cellular prion protein by dipeptidyl peptidase-4 and fibroblast activation protein

Andrew R. Castle[a,b,1], Sang-Gyun Kang[a,b], Ghazaleh Eskandari-Sedighi[a,c,2], Serene Wohlgemuth[a,b], My-Anh Nguyen[d,e], Daniel J. Drucker[f,g], Erin E. Mulvihill[d,e], and David Westaway[a,b,c,*]

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Keywords: beta-cleavage, dipeptidyl peptidase, DPP4, prion disease, prolyl endopeptidase FAP

Abstract

The cellular prion protein (PrP^C) converts to alternatively folded pathogenic conformations (PrP^{Sc}) in prion infections and binds neurotoxic oligomers formed by amyloid-β, α-synuclein and tau. β-Endoproteolysis, which splits PrP^C into N- and C-terminal fragments (N2 and C2, respectively), is of interest because a protease-resistant, C2-sized fragment ("$C2^{Sc}$") accumulates in the brain during prion infections, seemingly comprising the majority of PrP^{Sc} at disease endpoint in mice. However, candidates for the underlying proteolytic mechanism(s) remain unconfirmed *in vivo*. Here, a cell-based screen of protease inhibitors unexpectedly linked type II membrane proteins of the S9B serine peptidase subfamily to PrP^C β-cleavage. Overexpression experiments in cells and assays with recombinant proteins confirmed that fibroblast activation protein (FAP) and its paralog, dipeptidyl peptidase-4 (DPP4), cleave directly at multiple sites within PrP^C's N-terminal domain. For wild-type mouse and human PrP^C substrates expressed in cells, the rank orders of activity were human FAP ~ mouse FAP > mouse DPP4 > human DPP4 and human FAP > mouse FAP > mouse DPP4 >> human DPP4, respectively. C2 levels relative to total PrP^C were reduced in several tissues from FAP-null mice, and, while knockout of DPP4 lacked an analogous effect, the combined DPP4/FAP inhibitor linagliptin, but

not the FAP-specific inhibitor SP-13786, reduced $C2^{Sc}$ and total PrP^{Sc} levels in two murine cell-based models of prion infections. Thus, the net activity of the S9B peptidases FAP and DPP4 and their cognate inhibitors/modulators affect the physiology and pathogenic potential of PrP^{C}.

E

Prion-like Aggregation and Phase Transition of Mutant p53 as a Novel Cancer Treatment Strategy

Jerson L. Silva

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Biomolecular condensates are unique structures that form through liquid-liquid phase separation, without the presence of a membrane. While they serve essential physiological roles, they can also undergo a solid-phase transition, leading to amyloid-like structures that contribute to degenerative diseases and cancer. We will present recent research on the formation of biomolecular condensates and protein aggregates in cancer, with a particular focus on the tumor suppressor protein p53. Mutations in the *TP53* gene are found in more than half of malignant tumors and pose a significant threat to global health. Thus, it is crucial to understand the molecular mechanisms behind the loss of function (LoF), negative dominance (ND), and gain of function (GoF) of mutant p53, which all contribute to cancer development. Our studies have shown that not only does mutant p53 undergo misfolding, but it also forms biomolecular condensates and aggregates that resemble amyloids produced by other proteins. Interestingly, the mechanisms responsible for the GoF of mutant p53, much like toxic amyloids in neurodegenerative diseases, remain elusive. However, we have found that various cofactors, including nucleic acids and glycosaminoglycans, play a critical role in both classes of diseases. Moreover, molecules that inhibit mutant p53 aggregation have shown promise in reducing tumor proliferation and migration. Thus, phase transitions to solid-like amorphous and amyloid-like states of mutant p53 emerge as promising targets for the development of novel diagnostic and therapeutic strategies against cancer. (Supported by funds from CNPq, FAPERJ, CAPES and FINEP).

The prion protein from neurodegeneration to cancer

Sophie Mouillet-Richard

Centre de Recherche des Cordeliers, INSERM U1138 Sorbonne Université, Université Paris Cité, Paris, France

Ever since its discovery in the mid 80s', the prion protein calls to mind neurodegenerative diseases. The serendipitous observations that the prion protein (PrP) encoding gene *PRNP* is overexpressed in a pancreatic cancer cell line and upregulated in a drug-resistant versus parental gastric cancer cell line over 20 years ago have cast a new light on the disease-associated role of PrP. Yet, these findings have gone mostly unnoticed in the prion community, in stark contrast to the widening role of PrP in other brain proteinopathies, exemplified by Alzheimer's disease (AD).

In recent years, however, the link between PrP and cancer has shifted from a matter of curiosity to a research opportunity. PrP is now recognized as being overexpressed in a variety of cancer types, including breast, digestive and brain.

Using cell-based assays and patient cohorts, our laboratory has recently produced new insights into the contribution of overexpressed PrP to the poor-prognosis, mesenchymal subtype of colon cancer. Inspired by the signaling pathways associated with PrP that are hijacked in Alzheimer's disease, we have produced the proof of concept that targeting the interaction between PrP and AD-associated Aβ restrains tumor growth and hinders the development of metastases. Our global objective is to apply the knowledge gained on the pathophysiological role exerted by PrP to decipher its contribution to cancer progression and unfold new avenues for therapeutics.

Deranged synaptic signaling in experimental prion disease

Christina Sigurdson

University of California, San Diego

Synapse loss is an early feature of prion- and amyloid-β-induced neurodegeneration. To investigate the mechanisms of early synapse loss in prion disease, we have developed a knockin mouse model expressing amino-terminally mutated prion protein. Mice show an early onset of neurologic signs, including kyphosis, myoclonus, hind leg clasp, and seizures, together with widespread necrosis of hippocampal neurons and scattered spongiform change. We compare synaptic protein analysis of this genetic model to prion-infected mice at early disease stages with a focus on the cerebral cortex and hippocampus, toward a goal of identifying the initial synaptic response to prions.

Reshaping connectivity with tunneling nanotubes and role in the spreading of prion-like proteins in neurodegenerative diseases

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Tunneling nanotubes (TNTs) are newly discovered membranous connections between distant cells that enable the transfer of various cellular materials between them. We have shown that TNTs mediate the transmission of amyloid proteins involved in neurodegenerative diseases (NDs) (e.g., Alzheimer's, Parkinson's, Prion) between neurons. We postulate that TNTs are involved in the progression of these pathologies by facilitating the spreading of amyloid proteins throughout the brain, thus representing a novel therapeutic target to halt these incurable diseases. While TNTs may be involved in cell-to- cell communication in many physiological processes as well as diseases, their physiological relevance is unclear. Their fragility and lack of molecular markers make the observation of TNTs in vivo very difficult and has raised a lot of skepticism as to their physiological significance. Therefore, their structural and functional characterization and identification in vivo are absolutely necessary. In the lab we have undertaken a comprehensive approach by employing interdisciplinary tools to - i) identify specific pathways leading to TNT formation; ii) uncover their role in the progression of neurodegenerative diseases; iii) demonstrate their existence in vivo.
In my talk I will summarize some of the latest published and unpublished data from the lab on these topics.

Refined transgenetic approaches to characterize the host-range and adaptive potentials of emerging and established chronic wasting disease strains

Glenn C. Telling

Prion Research Center, Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, Colorado, USA

Animal modeling approaches to estimate prion host range properties, including strategies to assess zoonotic risks posed by emergent strains, customarily rely on transgenic overexpression and assessments of prion susceptibility following direct infection of the CNS. Notwithstanding the significant advances accrued from this approach, transgenic models suffer from multiple complications due to overexpression and an inability to accurately reproduce critical aspects of prion diseases, most particularly the importance of peripheral pathogenesis during intra- and inter-species prion transmission. We therefore employed a refined strategy in which were engineered gene-targeted (Gt) mice where expression of PrP coding sequences is regulated by *Prnp* control elements. Our findings show that the inefficient responses of previously-generated knock-in models were not the result of limited transgene (over) expression. Indeed, our experiences with Gt mice expressing physiologically controlled levels of deer or elk PrP reveal improved susceptibilities to established and emergent strains of chronic wasting disease (CWD). To a large degree, these improvements derive from the ability to recapitulate essential aspects of peripheral as well as CNS pathogenesis. Here we shall summarize our investigations of emergent European and established North American CWD strains using Gt mouse models, explore their highly diverse properties which contrasts the relatively limited and stable profile of North American CWD, and describe their unstable adaptive potentials including acquisition of novel lymphotropic properties.

J

Title: Understanding the seeding capability, transmissibility, and pathogenicity of prion.

Abstract: Prion is well known for its transmissibility, which can transmit the disease from an individual to another, and experimentally, from a diseased tissue to an experimental animal. The peculiar properties of prion transmission have made the physical nature of the agent a focal point of prion research. In the past two decades, a plethora of prion protein (PrP) aggregates have been generated with recombinant PrP expressed in bacteria. While all these aggregates possess in vitro seeding capability, only a fraction of them can induce aggregation of PrP expressed in animals, and an even smaller number are capable of eliciting genuine prion disease in wild-type animals. Our studies of in vitro generated recombinant prion have unequivocally established that the conformation of PrP determines its transmissibility and pathogenicity. In this presentation, results of these studies will be presented and the intricate relationship among seeding capability, transmissibility and pathogenicity of prion and prion-like proteins will be discussed.

Biomarkers for prion and neurodegenerative diseases

Inga Zerr

National Reference Center for human prion diseases, Dept. of Neurology, Göttingen, Germany

Biomarkers are biological characteristics that can be measured in tissue samples or fluids and used as a diagnostic tool for pathological changes or can also indicate physiological processes. Typically, parameters used as biomarkers in medicine are those that correlate with a particular pathology in either elevated or lowest concentrations. These are classically protein or peptides that can be assayed in abnormal concentrations (elevated or decreased) in body fluids, in neurological diseases predominantly in cerebrospinal fluid. A new class of aggregation assays (PMCA and/or RT QuIC) exploits the property of proteins to aggregate at increased levels when in pathological form. RT-QuIC for prion protein are now making their way into the clinical diagnosis of prion diseases. Human prion diseases were the first group of neurodegenerative disease to utilize both classical biomarkers and later aggregation assays to substantiate the suspected clinical diagnosis. Later this principle was also applied to other neurodegenerative diseases, so that in the meantime the concentration of tau, p-tau and A beta peptide are important components of the clinical criteria in Alzheimer's disease, in the field of synucleinopathies the synuclein aggregation assays are increasingly being researched. There are also now many more opportunities to study different body fluids to diagnose the disease. While CSF was a major component of many studies, there are now an increasing number of reports showing that other body fluids, such as plasma, serum, urine, sputum, and even tear fluids, can also be used for diagnosis.

Neurotherapeutics for Parkinson's disease.

Jeffrey H. Kordower, Ph.D. Founding Director, ASU-Banner Neurodegenerative Disease Center, Arizona State University Tempe AZ

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease and by 2040 it is believed that over 14 million individuals will suffer from PD worldwide. PD is defined clinically by a movement disorder characterized by bradykinesia plus either resting tremor, cogwheel rigidity and/or postural instability. These symptoms are due mainly to striatal dopamine insufficiency secondary to the degeneration of dopaminergic neurons in the substantia nigra. In addition, PD patients suffer from a myriad of non-motor symptoms that are believed to mediated by the spread of misfolded proteins especially alpha synuclein. This spread has been hypothesized to be initiated in the gut and olfactory system where it spreads caudally and rostrally respectively. This lecture will focus on a series of novel clinical and preclinical studies aimed at reducing or curing these symptoms as well as making progress towards disease modifying therapies. Pharmacological therapies aimed at enhancing the usefulness of levodopa by reducing side-effects while sustaining its therapeutic potency will first be discussed. Then experiments using antibodies and nanobodies aimed at preventing the spread of alpha-synuclein both preclinically and clinical will be described. The utility of deep brain stimulation and focused ultrasound will be described. Ongoing clinical trials describing gene delivery to provide trophic factor support with GDNF and preclinical studies aimed at reducing dyskinesias with CaV1.3 delivery will be discussed. Finally, the potential for cell replacement strategies using dopaminergic stem cells will be illustrated.

M

Sonia Vallabh
Prion 2023 abstract
Max 250 words
Due 3/31

Title: Lowering PrP: Evidence of therapeutic benefit across diverse yet relevant models.
Presenter: Sonia Vallabh
Authors: Meredith Mortberg, Juliana Gentile,Hien Zhao, Jill O'Moore, Deb Cabin, Holly Kordasiewicz, Eric Vallabh Minikel, Sonia Vallabh

Many lines of evidence support the proposition that lowering normal prion protein (PrP) levels would be therapeutically beneficial in prion disease, including genetic proofs of concept from PrP knockout and transgenic mice, and pharmacological evidence based on PrP-lowering antisense oligonucleotides (ASOs). Motivated by an ongoing series of discussions with regulators, we are pushing to illuminate the robustness of the link between PrP reduction and disease protection across prion disease models and paradigms. Understanding whether there exist circumstances under which PrP lowering would not, in fact, predict clinical benefit, can help us to best envision and tailor this biomarker's role in the drug development process. Using both ASOs and genetic perturbation of gene dosage, we have evaluated transgenic and knock-in mice expressing human PrP infected with human prions; rats and hamsters, representing relatively slow and fast models of prion disease; and a transgenic mouse model that develops spontaneous disease. Across all systems tested, we find that lower PrP corresponds to delayed disease. We will also share recent findings regarding cell-type distribution of ASO activity, with implications for how a a bulk target engagement biomarker such as PrP levels in cerebrospinal fluid may reflect drug activity across brain regions and cell types.

N

Prion meeting 2023

Title: Inhibition of prion like seeding of pathology in Parkinson's disease by antibodies and other therapies to alpha-synuclein in clinical development.

Pekka Kallunki, PhD, Principal Scientist, Protein aggregation, folding and clearance, H. Lundbeck A/S

Abstract:

The prion like seeding of pathology by alpha-synuclein aggregates has been widely reproduced in animal studies, where connectivity-mediated propagation throughout the brain in a prion-like manner is accompanied by neuronal dysfunction and degeneration. This has made alpha-synuclein a target of interest for antibody therapy and other therapies that could stop the uptake of alpha-synuclein seeds in neurons and/or clear the aggregates from brain. Recently, data from clinical trials testing two antibodies targeting α-synuclein in a population of patients with early Parkinson's disease (PD) reported negative results (Lang et al. 2022, Pagano et al. 2022). However, there was a lack of evidence of target engagement in these studies. Further, the evidence from preclinical models for inhibition of prion like seeding are limited for these two antibodies. Several other antibodies targeting α-synuclein are currently being tested in phase I and phase II studies in patients suffering from synucleinopathies, and other therapies targeting intracellular alpha-synuclein are progressing to clinic as well. This presentation covers the preclinical and clinical evidence for the new therapies that target alpha-synuclein and discuss if data suggest that they have a stronger treatment potential and better target engagement than previously tested antibody therapies.

Refs

Pagano G, Taylor KI, Anzures-Cabrera J, et al. Trial of Prasinezumab in Early-Stage Parkinson's Disease. N Engl J Med. 2022;387(5):421-432. doi:10.1056/NEJMoa2202867

Lang AE, Siderowf AD, Macklin EA, et al. Trial of Cinpanemab in Early Parkinson's Disease. N Engl J Med. 2022;387(5):408-420. doi:10.1056/NEJMoa2203395

O

Abstract for Prion 2023 Conference, October 16-20th, Faro, Portugal

Therapeutic Approaches for Alzheimer's Disease

Warren Hirst, Biogen

The global numbers of people with Alzheimer's disease (AD) have recently been estimated to be 32 million with AD dementia, 69 million with prodromal AD and 315 million with preclinical AD. AD is defined by its two pathological hallmarks: intracellular neurofibrillary tangles composed of aggregated and hyperphosphorylated tau and extracellular amyloid plaques the major component of which is the amyloid β-protein (Aβ), yet there are often additional pathologies, such as α-synuclein aggregates. These misfolded protein aggregates have characteristic 'prion-like' behaviors such as template-directed seeding, intra- and inter-cellular propagation, distinct conformational strains and protein-mediated toxicity.
Recent progress in soluble and imaging biomarkers has demonstrated that pathology precedes clinical manifestations, in some cases by multiple years or even decades, providing compelling rationale for early treatment. Given the complexity of the disease pathology in addition to treating as early as possible combination therapy is a logical next step.
Until recently, the only therapeutic options were symptomatic, i.e. not affecting the underlying disease progression. The recent FDA approvals of aducanumab and lecanemab, which target and remove Aβ, have not been without controversy, but do provide an option for patients and further motivation for research and development of additional therapeutics. These include antibodies, antisense oligonucleotides and small molecules targeting Tau; agents targeting TREM2 and other inflammatory mediators, metabolic pathways and ApoE4, which has the strongest impact on risk of late-onset AD. The merits and limitations of these various approaches will be discussed.

Word count: 237
Word limit: 250

Plasma TIA-1: A novel biomarker for Alzheimer's disease

Abrar Younas[a,b#], Neelam Younas[a,b#], Peter Hermann [a,b], Inga Zerr [a,b]

[a]Department of Neurology, Georg-August University, Goettingen, Germany; [b]German Center for Neurodegenerative Diseases (DZNE), Göttingen, Germany).

#: Equal contribution

Aims:

Early diagnosis of Alzheimer's disease and measurement of its invasiveness remains an obstacle. Emerging evidences indicate that an RNA-binding protein, T-cell intracellular antigen 1 (TIA-1) plays a key role in tau pathophysiology and toxicity. Reduction of this protein in the human brain of AD patients suggests a downregulation of TIA-1 as a protective mechanism. There is an urgent need to find out biomarkers that reveal pathological changes prior to the appearance of traditional biomarkers of neurodegeneration. To this end, we sought to explore the levels of TIA-1 in blood plasma in healthy control and AD subtypes (classical AD, rapidly progressive AD: rpAD).

Material and Methods:

We analyzed blood samples (n=43) from healthy individuals (n=14) and AD subtypes (n=29). Samples were assayed using the ELISA kit from Biobool (#E027072) according to the manufacturer's instructions. To check the normality of the values, Shapiro-Wilk normality test was used. In the cross-sectional analysis, the mean level of TIA-1 was compared among the three groups: control, ''classical'' AD, and rpAD by using a one-way analysis of variance with Tuckey post hoc comparison.

Results:

Plasma TIA-1 levels were significantly reduced in patients with classical AD (p-value < 0.0001) and rapidly progressive AD (p-value < 0.0001) compared with healthy control group. To determine the diagnostic accuracy of plasma TIA-1, we combined the classical and rapidly progressive AD groups. The ROC curve analysis (receiver operating characteristic) indicated plasma TIA-1 with AUC (area under the curve) values 0.9581 ± 0.04136 ($P < 0.0001$), suggesting TIA-1 as a promising marker to distinguish AD patients from healthy controls.

Conclusions:

Our exploratory study demonstrates the biomarker potential of TIA-1 for distinguishing AD subtypes from healthy individuals with high accuracy. To our knowledge, this is the first study to report regulation of TIA-1 in blood plasma in AD pathology. Future validations in larger independent cohorts will be important.

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	**x**
Therapeutic approaches for prion and prion-like diseases	

PRION 2023 abstract title: **"Combined diagnostic accuracy of MRI and CSF RT-QuIC in a large cohort of autopsy-confirmed prion disease patients"**

Riccardo Pascuzzo[1], Lavista Osborn[2], Keisi Kotobelli[2], Mark L. Cohen[2,3,4], Xiaoqin Liu[2], Jody Lavrich[2], Jaime Noguez[2,3], Shashi Shetty[2,3], Lawrence B. Schonberger[5], Pierluigi Gambetti[3], Brian S. Appleby[2,3,4,6], and Alberto Bizzi[1]

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[4] Department of Neurology, Case Western Reserve University, University Hospitals Cleveland Medical Center, Cleveland, Ohio, USA
[5] National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA
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Aim: To evaluate the combined diagnostic accuracy of MRI and CSF second generation Real-Time Quaking-Induced Conversion (RT-QuIC) in a large cohort of prion disease patients.

Materials and Methods: Patients enrolled by the NPDPSC were included if they had (1) autopsy-confirmed diagnosis (either prion or non-prion disease) and (2) RT-QuIC result and/or brain MRI study with diffusion-weighted image (DWI). An expert neuroradiologist, blind to diagnosis and RT-QuIC results, prospectively evaluated the DWI of all patients.

Results: **DWIs** were available in 1094 patients (923 with and 171 without prion disease): 785 had sporadic Creutzfeldt-Jakob disease (sCJD), 98 genetic CJD (gCJD), and 40 other prion diseases. DWI had excellent specificity (95.3%, 163/171) and sensitivity for sCJD (94.8%, 744/785), without significant differences among sCJD subtypes (90%–100%, p=0.3785). DWI sensitivity was good for gCJD (75.5%, 74/98). DWI negative predictive value (NPV) was 64.9% (163/251).
RT-QuIC results were available in 924 patients (819 with and 105 without prion disease), including 747 sCJD, 53 gCJD, and 18 other prion diseases. RT-QuIC had excellent specificity (98.1%, 103/105) and good sensitivity for sCJD (89.7%, 670/747) and gCJD (83.0%, 44/53). RT-QuIC sensitivity significantly differed among sCJD subtypes (p<0.0001), with the lowest values for VV1 (37.5%, 3/8) and MM2C (65%, 13/20), and the highest for MV2K (100%, 15/15) and VV2 (95.2%, 79/83). RT-QuIC NPV was 52.6% (103/196).
DWI and RT-QuIC were available in 210 sCJD, 22 gCJD, nine other prion diseases, and 24 non-prion disease patients. Combining the two tests, sensitivity increased for all forms of prion diseases, up to 100% in sCJD. Combined specificity was 83.3% (20/24). Combined NPV reached 80.0% (20/25).

Conclusions: Diffusion MRI and RT-QuIC are accurate and complementary tests for the *antemortem* diagnosis of prion diseases. In patients with suspected prion disease, if one test is negative, it is strongly recommended to perform the other test.

Funded by: **CJD Foundation** (CJD Foundation Grant 2021, Strides for CJD Grant, Walter Williams Memorial Research Grant, Sherry Maxwell Fabian Memorial Grant, Jeffrey A. Smith Memorial Research Grant) and **CDC** (Grant Number: NU2GCK000434).

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
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Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	X
Therapeutic approaches for prion and prion-like diseases	

Attempted Transmission of Chronic Wasting Disease from White-Tailed Deer to Sheep Leads to Non-Adaptive Prion Amplification (NAPA)

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[b]Virus and Prion Research Unit, National Animal Disease Center, USDA-ARS, Ames, IA, USA

[c]Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, Tennessee, USA

Aims: The purpose of this study was to investigate the interspecies transmission of the chronic wasting disease (CWD) agent between sheep and cervids.

Materials and Methods: Suffolk sheep (n=7) of various *PRNP* genotypes (VRQ/ARQ, ARQ/ARQ, and ARQ/ARR) were oronasally inoculated with the CWD agent (10% w/v brain homogenate, 1 mL) from a white-tailed deer with *PRNP* genotype GG96. Positive retropharyngeal lymph node (RPLN) material (20 µL of 10% homogenate) from 1/7 sheep (ARQ/ARQ) was then passaged twice through ovinized (Tg338) and cervidized (Tg12) mice. EIA (enzyme immunoassay) was performed on brain tissues to analyze PrP^{Sc} accumulation.

Results: Of the original 7 inoculated sheep, the only evidence of infection was detection of PrP^{Sc} in the RPLN of one sheep. There was no evidence of infection upon first passage to Tg338 mice, but PrP^{Sc} was detected in Tg12 mice (5/9 positive) with an average incubation period of 565 dpi (days post inoculation). On the second passage, no PrP^{Sc} was detected in mice that received inoculum from Tg338 mice. The second passage of material between Tg12 mice led to a 100% attack rate (13/13) with an average incubation period of 141 dpi.

Conclusions: After oronasal exposure, there was evidence of CWD infection in a single sheep suggesting there is not an absolute species barrier. As the sheep had PrP^{Sc} accumulation in lymphoid tissue, environmental shedding is a possibility. Subsequent passages in mice expressing ovine *PRNP* did not lead to detection of the CWD agent, but passages in mice expressing cervid *PRNP* recovered the CWD agent. While sheep did not develop clinical disease of CWD, they were capable of transmitting the agent back to cervidized mice demonstrating that this is an example of NAPA.

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Grant number: N/A

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Theme	
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
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Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	(X)
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Detection of classical BSE prions in asymptomatic cows after inoculation with atypical/Nor98 scrapie

Marina Betancor1, Belén Marín1, Alicia Otero1#, Carlos Hedman1, Antonio Romero2, Tomás Barrio3, Eloisa Sevilla1, Jean Yves Douet3, Alvina Huor3, Juan José Badiola1, Olivier Andréoletti3, Rosa Bolea1.

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3 UMR École Nationale Vétérinaire de Toulouse (ENVT) - Institut National pour l'Agriculture, l'Alimentation et l'Environnement (INRAE) - 1225 Interactions Hôtes Agents Pathogènes (IHAP), 31300 Toulouse, France.

Aims: The emergence of bovine spongiform encephalopathy (BSE) prions from atypical scrapie has been recently proved in rodent and swine models. This study aimed to assess whether the inoculation of atypical scrapie could induce BSE-like disease in cattle.
Materials and Methods: Four calves were intracerebrally challenged with atypical scrapie. Animals were euthanized without clinical signs of prion disease between 7.2 and 11.3 years post-inoculation and tested for the accumulation of prions by conventional techniques and protein misfolding cyclic amplification (PMCA).
Results: None of the bovines showed signs compatible with prion disease. In addition, all tested negative for PrP^{Sc} accumulation by immunohistochemistry and western blotting. However, an emergence of BSE-like prions was detected during in vitro propagation of brain samples from the inoculated animals.
Conclusions: These findings suggest that atypical scrapie may represent a potential source of BSE infection in cattle.

Funded by: This work was supported financially by the following Spanish and European Interreg grants: Ministerio de Ciencia, Innovación y Universidades (Spanish Government), cofunded by Agencia Estatal de Investigación and the European Union and POCTEFA, which was 65% co-financed by the European Regional Development Fund (ERDF) through the Interreg V-A Spain-France-Andorra program (POCTEFA 2014–2020).
Grant number: n° PID2021-125398OB-I00, EFA148/16 REDPRION

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Theme	(X)
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Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Adaptation of diverse, non-lymphotropic Nordic CWD isolates to lymphotropic strains following serial passage in cervidized mice

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[1]Department of Microbiology, Immunology, and Pathology, Colorado State University, United States; [2]National Veterinary Institute, Sweden; [3]Department of Biomedical Sciences and Veterinary Public Health, Sweden [4]Norwegian Veterinary Institute, Norway

Aims: Chronic wasting disease (CWD) is a highly contagious prion disease affecting cervids in North America (NA) and, more recently, Europe. Characterization of emergent CWD in Norway, Sweden, and Finland indicate these isolates differ from NA CWD. Nordic CWD isolates are more diverse than stabilized NA CWD strains and are non-lymphotropic, with the exception of CWD in Norwegian reindeer. Studies of Nordic CWD in gene-targeted (Gt) mice, which express physiological levels of cervid PrP with either glutamine (Q) or glutamate (E) at residue 226, recapitulate the lymphotropism of natural CWD isolates. Transmission studies of the second Norwegian moose CWD isolate (M-NO2) found M-NO2 preferentially infects GtQ mice and is non-lymphotropic. However, transmission through GtE mice resulted in isolation of a strain that more efficiently transmits to the E background and became lymphotropic, resembling NA CWD isolates. To investigate if other non-lymphotropic Nordic CWD isolates undergo a similar adaptation process during serial passage in Gt mice, lymphoid tissue was evaluated for presence of PrP^{Sc}.

Materials and Methods: Field isolates from Norwegian and Swedish CWD-infected cervids were intracranially (i.c.) inoculated into GtQ and GtE mice and serially passaged. Presence of PrP^{Sc} in spleens from Gt mice infected with Nordic isolates was evaluated by Western blot and confirmed by real-time quaking-induced cyclic amplification (RT-QuIC). To analyze neuropathology, brain sections underwent immunohistochemistry to assess PrP deposition.

Results: Western blot of spleen homogenates from three isolates—a Norwegian red deer, Norwegian moose, and Swedish moose—revealed the presence of PrP^{Sc}, indicating acquisition of lymphotropism during serial transmission. Interestingly, these isolates also share a distinct PrP deposition pattern in the dentate gyrus, which is overwhelmingly observed when passaged in the GtE background.

Conclusions: Combined, these data suggest adaptation of diverse, unstable Nordic CWD isolates towards a lymphotropic CWD strain facilitated by passage in the E background, potentially leading to an adaptation event similar to M-NO2.

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and Stroke

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	X
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Bloodborne prions detected in healthy-appearing CWD-infected free-ranging white-tailed deer in several U.S. states

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Aims:
The infectious agent that initiates prion infection has been demonstrated in the blood of humans, nonhuman primates, sheep and cervids. Yet, the development of rapid, sensitive, and specific assays to detect prions in blood has been challenging due to presumed low concentrations of hematogenous prions and/or inhibitors present in blood. We have capitalized on a unique and extensive repository of serially-collected blood samples from longitudinal CWD experimental studies in the native white-tailed deer (WTD) host, collected minutes post CWD exposure through terminal clinical CWD presentation, to demonstrate the presence of bloodborne prions by lipase iron-oxide bead RT-QuIC (LIQ). Here we aim to explore LIQ's ability to detect prions in the blood of WTD naturally-exposed to CWD.
Materials and Methods:
Field-collected blood samples from free-ranging WTD in CWD endemic areas of Arkansas, Tennessee and West Virginia were preserved in ACD tubes, held for 3-5 days at refrigerated temperatures and shipped to CSU. Buffy coat cells (BC) were extracted from each sample and processed by LIQ.
Results:
We found that white blood cells routinely retain integrity for successful isolation after several days held at refrigerated temperatures, and as little as 1mL of anticoagulated whole blood contained sufficient BC for LIQ. In addition, this study reveals a correlation between the stage of CWD infection and prion detection in BC. We demonstrate prions in the blood of the majority of lymphoid positive deer (RAMALT 73%, tonsil 80%, and retropharyngeal 85%), and 100% of deer with CNS involvement.
Conclusions:
We have optimized the RT-QuIC assay to detect bloodborne prions using lipases and iron-oxide bead extraction and have demonstrated detection of prions in blood collected in early to late-stage disease from both experimental and natural CWD-infected WTD. Future work will explore the potential of this assay in diagnostics and surveillance of additional free-ranging cervid populations.

Funded by: Multistate Conservation Grant Program, The Association of Fish and Wildlife Agencies and HHS NIH NIAID
Grant numbers: F20AP00172 and HHS NIH NIAID R01AI112956

Acknowledgement: We thank our collaborators in Georgia, Tennessee, West Virginia and Arkansas for collecting and providing us with samples from free-ranging white-tailed deer.

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	(x)
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF PRIMARY CORTICAL NEURONS DEVOID OF THE PRION PROTEIN

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AIM

The cellular form of the prion protein (PrP^C) is a ubiquitously expressed protein playing a fundamental role in the progression of prion diseases. It also exerts a plethora of physiological functions: we hypothesize that its expression could modulate neuronal excitability and synaptic transmission. Our aim is to observe and quantify electrical activity alterations in *ex vivo* primary cultures of PrP^C knock out cells. Moreover, acting as a receptor for protein aggregates involved in neurodegenerative diseases, we will investigate the electrophysiological phenotype of primary neurons exposed to aggregates of recombinant proteins such as tau, α-synuclein and TDP-43.

MATERIALS AND METHODS

We employed substrate-integrated MicroElectrode Arrays (MEAs) to non-invasively detect the extracellular electrical activity of networks of cultured primary neurons, dissociated from the murine cortex (FVB, P0/P1; WT, $PrP^{+/+}$, KO, $PrP^{-/-}$).

RESULTS

Preliminary evidence reveals differences between $PrP^{+/+}$ and $PrP^{-/-}$, both in terms of duration and occurrence rate of episodic spontaneous synchronous network electrical events, when the culture reached maturity (~23 days *in vitro*, DIVs) and at earlier time-points (10 and 17 DIVs, during *ex vivo* maturation). Finally, dissecting whether PrP^C involvement in neuritogenesis or synaptogenesis might explain our electrophysiological data, we observed a different number of synapses upon PSD-95 staining over time.

CONCLUSION

The difference in the rate of episodic synchronization of the neuronal activity between $PrP^{+/+}$ and $PrP^{-/-}$ mice is consistent with our hypothesis on the physiological role of the prion protein: if knocked out, the network-level electrical activity becomes disrupted.

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	X
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Expression of the cellular prion protein by mast cells in white-tailed deer carotid body, cervical lymph nodes and ganglia

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Aims: The structure and function of the carotid bodies places them in a unique position to be involved in the pathogenesis of prion diseases. The aims of this study were to determine if cells of the white-tailed deer carotid body express the prion protein.

Materials and methods: Blocks of tissue containing the carotid body were collected from white-tailed deer and immersion fixed prior to embedding in paraffin. Tissue sections were cut on a microtome (7µm) and stained using hematoxylin and eosin to identify the carotid body (CB). Tissue sections containing CBs were stained with toluidine blue (TB) to identify mast cells and the prion antibody, 8H4, was used to identify cells expressing the prion protein.

Results: Mast cells were identified in CBs collected from every deer (n=9). Mast cells are most often found within connective tissue capsules that surrounds the CBs and within connective tissue fascicles that separate clusters of CB cells. The morphology and distribution of cells expressing the prion protein as determined by using the 8H4 antibody was the same as mast cells that were identified using TB. 8H4-positive cells that were counterstained with TB confirmed that mast cells in the CB express the prion protein. Lymph nodes and superior cervical ganglia that were included in these tissue blocks were also noted to contain mast cells that express the prion protein.

Conclusions: Mast cells located in the CBs would be exposed to infectious prions circulating in the blood of infected subjects and could be involved in the spread of infectious prions to the brainstem and thoracic spinal cord as CBs are innervated by nerves that are synaptically linked to these areas, which are known targets of neuroinvasion following prion exposure.

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Prion 2023 - Combined analyses of peripheral tissues collected from patients with Parkinson's disease and subjects with isolated REM sleep behavior disorder

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Aims
We have analyzed the olfactory mucosa (OM) and serum samples of patients with Parkinson's disease (PD) and individuals with isolated REM sleep behavior disorder (iRBD) with a multidisciplinary approach. In particular, we have (i) assessed the distribution of disease-associated α-synuclein (αSyn^D) in OM samples, (ii) analyzed the nasal microbiota composition and its potential role on αSyn^D strain formation, (iii) evaluated the NfL levels in OM and serum samples. The main aim of the study was to investigate the validity of our approach to generate specific PD and iRBD biological fingerprints.

Materials and methods
We enrolled PD (n=18), iRBD (n=10) and healthy subjects (n= 16, HS) who underwent olfactory evaluation, OM and blood collection. OM were collected from left and right nostrils and were analyzed separately. Seed Amplification Assay (SAA), ELLA™ and MiSeq Illumina were employed to determine αSyn^D distribution, NfL levels and microbiota composition, respectively, in OM. ELLA™ was used to measure the serum levels of NfL.

Results
SAA showed a higher seeding activity in OM of iRBD samples than PD. Interestingly, the majority of the seeding activity was sustained by the left OM. Interestingly, there was no correlation between seeding activity and the severity of olfactory impairment or disease stage. Serum levels of NfL were significantly higher in both iRBD subjects and PD patients

compared to HS. Conversely, the NfL levels measured in OM did not correlate with those in the serum. Nasal microbiota analysis is complete and statistical analyses are in progress.

Conclusion
αSyn^D can be detected in OM of iRBD patients with higher efficiency than PD. This suggests that iRBD may represent a more severe form of α-synucleinopathy. In addition, our combined approach has the potential to generate PD and iRBD biological fingerprints eventually useful to identify iRBD subjects at higher risk of developing α-synucleinopathies.

Funded by
This work was supported by the Italian Ministry of Health to Fabio Moda

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Acknowledgement
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Theme: Biomarkers for prion and other neurodegenerative diseases

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	X
Therapeutic approaches for prion and prion-like diseases	

Microglia-specific IKK and NF-κB signaling in prion infection

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Aims: The role of glial cells in prion disease pathogenesis and neurotoxicity is poorly understood. Microglia can phagocytose PrP^{Sc}, leading to the release of inflammatory signaling molecules, which subsequently induce astrocyte reactivity. Animal models show highly upregulated inflammatory molecules that are a product of the Nuclear Factor-kappa B (NF-κB) signaling pathway, suggesting that this is a key regulator of inflammation in the prion-infected brain. The activation of the IKβ kinase complex (IKK) by cellular stress signals is critical for NF-κB-induced transcription of a huge variety of genes, including inflammatory cytokines and chemokines, enzymes, and genes involved in cell survival and autophagy. However, the contribution of microglial IKK and NF-κB signaling in the prion-infected brain has not been evaluated. Here, we assess the role that microglial IKK and NF-κB signaling has on disease pathogenesis.

Materials and Methods: We characterized prion infection in a transgenic mouse model containing wild-type (WT) astrocytes and IKK knock-out (KO) microglia and in primary mixed glial cells derived from these mice. Mixed glial cells were infected with prions and analyzed for NF-κB-associated genes and prion accumulation. We evaluated clinical and behavior signs of prion disease in these mice. We compared neuron, microglia and astrocyte numbers and morphology, PrP^{Sc} deposition, and spongiosis between these animals and WT mice.

Results: Upon infection with prions, NF-κB-associated genes are significantly downregulated in mixed glial cultures containing IKK KO microglia. Prion infected mice with IKK KO microglia show rapid disease progression, including an increase in microglia and reactive astrocytes, and accelerated loss of hippocampal neurons and associated behavioral signs. These animals display clinical signs and have a 25% shorter life expectancy compared to infected wild-type mice. Moreover, PrP^{Sc} accumulation was found to be significantly increased in primary mixed glia containing IKK KO microglia, as well as in the brains of infected animals with IKK KO microglia.

Conclusions: These findings present a critical role in IKK and NF-κB signaling from microglia in host protection, and may uncover both biomarkers and therapeutic targets for prion diseases.

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Acknowledgements: Special thanks to McKenzie Richards, Amanda Latham, and Casey McDermott for their help and guidance, and the mice that dedicated their lives to this research

Theme: Pathogenic mechanisms in prion and prion-like diseases

Chronic wasting disease *in utero* transmission in free-ranging white-tailed deer

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Aims:
The transmission of chronic wasting disease (CWD) within cervid populations has been largely attributed to horizontal transmission via direct (animal-to-animal) or indirect (shed prions in the environment) contact. Previous studies in free-ranging white-tailed deer (WTD), Rocky Mountain elk, and experimental studies in Reeves' muntjac deer have demonstrated that mother-to-offspring transmission (vertical transmission) is another likely source of infection. To expand on this research, we investigated the role *in utero* transmission plays in free-ranging WTD with naturally acquired infections from three states in the southeastern United States.

Materials and Methods:
Maternal and in utero-derived fetal tissues (n=56) were harvested from healthy appearing dams (n=31) in CWD endemic areas of Arkansas, Tennessee, and West Virginia as well as one state with no known CWD infections (Georgia) for comparison. Tissues were assessed for amyloid seeding activity using real time quaking induced conversion (RT-QuIC), immunohistochemistry (IHC), protein misfolding cyclic amplification (PMCA), and/or iron-oxide bead extraction (IOB). Furthermore, to determine the biological relevancy of these samples, a transgenic cervid mouse bioassay was initiated to assess the presence of infectivity in maternal and fetal tissues.

Results:
Of the 17 CWD+ dams, we found CWD prions in the maternal reproductive tract (uterus, placentomes and amniotic fluid) of 8 dams and within *in utero*-derived fetal tissues (brain and thymus) harvested from 5 dams, as assessed by RT-QuIC. This provides evidence for fetal CWD exposure prior to parturition. To further determine the relevance of these findings, mouse bioassay revealed the presence of infectivity in both maternal reproductive tissues and fetal tissues.

Conclusions:
This study supports earlier experimental findings, and for the first time demonstrates CWD mother-to-offspring transmission in free-ranging white-tailed deer. Continued exploration of CWD transmission dynamics and associated mechanisms from mother-to-offspring provides further understanding of the facile and efficient transmission of CWD in the native cervid host.

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Grant number: F20AP00172 and NIH NIAID R01I093634

Acknowledgement:

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	(x)
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Differentiation of Chronic Wasting Disease strains in Texas and Missouri

Authors: Avery Lessard[1,3], Kaitlyn Wagner[1,3], Arielle Hay[1,3], Analeis Cofield[1,3], Julie A. Moreno[2,3] and Mark Zabel[1,3]

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Chronic Wasting Disease (CWD) is a Prion disease that targets cervid species. Today, CWD has been reported in at least 26 states across the United States. The disease has even been linked to some areas in Canada, South Korea, and Nordic countries. We have previously shown that prion isolates can present with different strains.

Aims: In this study we aim to compare prion isolates from different states to determine if there is evidence to support strain differences.

Materials and Methods: To address this, we inoculated transgenic mice with brain homogenates from infected deer from Texas (3) and Missouri (11). We employed behavioral assays during infection until the mice succumbed to the disease. Histological and biochemical tests were performed on the brains extracted from the euthanized mice.

Results: We observed significant behavioral, histological, and biochemical differences among prion isolates from Missouri and Texas.

Conclusions: This work identified specific phenotypic differences among various CWD prion isolates that support our conclusion that these cervids propagate unique prion strains.

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Grant number: F22AP00153

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Theme	(X)
Neuropathology of prion diseases	x
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	x
Animal prion diseases	x
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Prion 2023 - **Chaperones: A Eukaryotic Amyloid Defence System**

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Aims: Chaperones have become a promising therapeutic target in neurodegenerative disorders such as Alzheimer's and prion diseases as these have been implied in the fragmentation of causal protein aggregates. The fragmentation of these amyloids can lead to two different outcomes, namely the dissolution of the aggregates or the fragmentation and propagation of seeds. Therefore, a comprehensive understanding of chaperone fragmentation is vital, yet the mechanisms behind the fragmentation of amyloid aggregates and the interaction among different chaperones to achieve this fragmentation still remains elusive. To gain this crucial insight into their mechanisms and interplay, *Saccharomyces cerevisiae* is a bona fide model organism. *S.cerevisiae* expresses an endogenous translation termination protein, Sup35, which can form prion [PSI+] that is modulated by a chaperone complex, Hsp104-Ssa1-Sis1.

Materials and Methods: Currently, we are on the verge of resolving the structure of this yeast prion and chaperone binding using single-particle cryo-EM. Furthermore, due to the ease of genomic mutations in yeast, we will introduce endogenous fluorescent markers to explore the disaggregation of amyloid fibrils *in situ* using a cryogenic correlative light and electron microscopy (cryo-CLET) workflow. Additionally, super resolution microscopy will be used to investigate the kinetics of Sup35 fibrilization but also of chaperone fragmentation of this prion protein.

Conclusions: These workflows will produce high-resolution structural and kinetics studies of this intrinsic amyloid defence system in eukaryotes that will be pivotal in fighting amyloid diseases.

Funded by: Medical Research Council

Grant number: 185931

Acknowledgement: Ziang Wang, Ryan Buckley, Mark Batchelor

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	X
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Stored losses? A curious case of the disappearing prion protein in formalin-fixed atypical scrapie tissue.

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Aim: APHA has a world-leading bioarchive with a collection of TSE-related tissues from 1987 till date. These tissues are routinely stored in formalin-based fixatives. A recent observation of a decline in or total loss of positivity of retrospective atypical scrapie tissues stored in formalin-based fixatives lead us to posit that the fixatives could be oxidising into formic acid – a potent prion protein denaturant with implications for the integrity of such important samples.

Method: Using a formic acid quantification kit, the amount of formic acid in formalin-based fixatives dating from 2000 until 2023 was quantified. For each year within this period, aliquots of formalin-based fixatives of three different TSE-negative samples were obtained. A graph of the amount of formic acid against the year was plotted for the samples. Serial sections of an atypical scrapie case were randomly treated with a time course of formic acid to determine the impact of prolonged formic acid treatment on tissues. Prion protein positivity was assessed by immunohistochemistry.

Results: We observed an overall negative correlation between age of the fixative and formic acid levels. A negative correlation between the atypical scrapie IHC intensity and the duration of formic acid treatment was observed.

Conclusions: Oxidation of formalin-based fixatives into formic acid could be implicated in the loss of atypical scrapie signal in retrospective positive cases through denaturation of the prion protein. Further work is required in understanding the mechanism to identify possible alternatives to formalin-based fixatives.

Funded by: DEFRA

Grant number: SE1962

Theme: Animal prion diseases

Prion 2023: Classical BSE in Great Britain: Review of its epidemic, risk factors, policy and impact

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Aims: To provide a current and historical perspective of the epidemiological pattern of classical BSE (cBSE) in Great Britain (GB), the policies implemented, its impact and the risk factor associated to BARB (born after the reinforced ban) cases.

Material and Methods: The study used cBSE data collected from 1986 to 2021 in GB for the epidemiological analysis, to compare recent and historical cases and to assess spatio-temporal clustering of classical BARB cases. Risk factors for BARB cases were reviewed using scientific literature via PubMed and epidemiological reports. The range of policies and impact of BSE were reviewed through consultation of national and international policy documents and grey literature.

Results: To date, a total of 181,122 cBSE cases have been found in GB, of which 178 are BARB cases (0.1%). The number of cBARB cases steadily declined post-2003 and only two cases were detected between 2016 to 2021 (0.29 cases per 100,000 cattle). Feed-borne route was found as the most likely source of infection. Although a small spatial cluster was found, overall findings reinforce the hypothesis that cases are not generated spontaneously, but major gaps remain on their aetiology.

BSE transformed the industry and our approach to animal health. The disease caused large economic impact through disposal of animals and products, reduction of the national herd, trade losses, market prices disruptions, costly cleaning and control procedures and implementation of expensive government strategies. Yet, the disease generated many positive developments, from new technologies to more robust traceability systems.

Conclusion: The review provides an insight into the shock that a novel disease such as BSE could have on the society. Numerous lessons learnt have improved country's preparedness to future diseases. As we work towards the eradication, the story of BSE is a clear example of how to control a disease under major uncertainties.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	X
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Early and Terminal Detection of Chronic Wasting Disease in Retropharyngeal Lymph Node Cell Subsets

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Aims: Chronic Wasting Disease (CWD) is known to have significant lymphoreticular system involvement. The presence of prions in blood cell components of CWD infected cervids is detected as early as 15 minutes post inoculation (pi). Yet, the presence of prions within specific lymphoid tissue cell subsets remains relatively understudied. To gain better understanding of the peripheralization of blood-borne prions, we analyze specific retropharyngeal lymph nodes (RLN) cell populations during early and terminal CWD infection for their prion carrying capacity.

Materials and Methods: *Early CWD infection:* n=6 white-tailed deer (WTD) were intra-nasally-inoculated with 0.5 mg CWD+ brain. The deer were sacrificed at 1, 2, or 3 months pi (n=2 each) and RLN were collected. *Terminal clinical CWD:* n=8 WTD were orally inoculated with 10mg CWD+ brain (n= 4) or 10mg CWD+ brain bound to montmorillonite clay (n=4). CWD- inoculated deer were also sacrificed at each time point (n=5).

Cell populations (B cells, CD4 T cells, and CD8 T cells) were magnetically separated from RLN, were confirmed/assessed for purity by flow cytometry, and analyzed for CWD status by Real Time Quaking Induced Conversion.

Results: At 1-month pi, all LN specific cell populations and whole RLN remained CWD- by RT-QuIC. At 2 and 3 months pi, prion detection was demonstrated in all RLN cell populations and whole dissociated RLN. Cell population data from clinical animals is currently being generated and will be included in poster results.

Conclusions: Our findings suggest that prions traffick within the blood to RLN between 1 and 2 months pi. Further assessment of blood and lymphoid cell subsets will contribute to our understanding of early intra-host prion trafficking and reveal the role of specific cell subset contributions to prion pathogenesis. Furthermore, these findings will enhance our ability to mitigate CWD and other protein misfolding disorders.

Funded by: NIH

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	X
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Adapting tissue processing for western-blot detection of PrP^{Sc} in fixed tissues

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Aims

Western blotting is a widely employed technique for detecting and characterizing proteins in biological samples and it is one of the methods recommended by the WOAH for the confirmatory diagnosis of animal TSEs. While traditionally used with fresh tissues, the application of western blotting to aldehyde fixed tissue has gained significant attention in recent years. Formaldehyde fixed tissue offers several advantages, including improved sample stability and preservation of tissue morphology whilst also inactivating a broad range of pathogens which is especially relevant when handling veterinary specimens where co-infection, most notably with *Mycobacterium bovis*, is not infrequent. However, working with fixed tissue poses certain challenges. Formaldehyde fixation involves the formation of cross-links between proteins which may alter protein conformation, potentially affecting the antibody recognition and antigen accessibility required for western-blotting techniques. Here we describe a tissue processing protocol which allows the removal of fixative to expose the target epitopes on PrP^{Sc} and allow subsequent detection by western blot.

Material & Methods

Formalin fixed brain tissue samples from classical and atypical scrapie infected sheep and classical bovine spongiform encephalopathy (BSE) infected cattle were washed with 95% ethanol and PBS. Tissues were subsequently homogenised in lysis buffer containing a formaldehyde scavenger agent and ionic detergent and incubated at 95°C for 2 hours before re-homogenisation. Subsequently they were processed according to the standard APHA Bio-Rad Hybrid western blot protocol.

Results

The adapted method allowed us to observe the classic banding pattern associated with protease treated PrP^{Sc} from ovine and bovine TSE infected tissue from fixed material with little reduction in sensitivity when compared to the unfixed equivalents.

Conclusion

The stringent formaldehyde removal treatment included in our new protocol is successful to release PrP^{Sc} from formaldehyde fixed tissues allowing subsequent detection by western blot. This will expand our TSE diagnostic capability and enable us to conduct western blot testing in cases where only fixed tissue samples are available and the histology-based tests cannot give a conclusive result.

Acknowledgements

We would like to thank the Biological Archive team at APHA for providing the tissues for this study.

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Theme: Animal Prion Diseases

Cellular disaggregation and degradation systems generate seeding and spreading-competent α-Syn and Tau species

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Aims:

Molecular chaperones play a vital role in maintaining cellular protein homeostasis (proteostasis) and have the potential to interfere with the pathological progression of α-Syn and Tau aggregates. This study was part of a JPND collaboration aiming to identify and characterize components of the Hsp70/co-chaperone network that influence the aggregation, spreading, and toxicity of α-Syn and Tau.

Materials and Methods:

This work involved *in vitro* biochemistry, *C. elegans* and cell culture models to study α-Syn and Tau aggregation, spreading and toxicity.

Results:

The Hsp70 disaggregase, a combination of the constitutively expressed Hsc70, the J-domain protein DNAJB1 and the nucleotide exchange factor (NEF) Hsp110 can disaggregate recombinant α-Syn and Tau fibrils *in vitro*. However, chaperone-mediated disassembly of amyloid fibrils generated smaller fragments with increased seeding capacity. Moreover, Knockdown of HSP-110, an essential component of Hsp70 disaggregase, reduced the formation of toxic and spreading-competent α-Syn species in a *C. elegans* model system. At the same time, the reduction of HSP-110 prevented the disaggregation of amorphous aggregates and impaired the overall cellular folding capacity. These data suggest that HSP70 disaggregation activity is a double-edged sword, as it is important for the maintenance of cellular proteostasis but is also involved in the formation and propagation of toxic protein species.

To further understand the role of cellular protein quality control pathways in the seeding and propagation of α-Syn, fluorescence lifetime imaging microscopy (FLIM) was employed to investigate seeded aggregation of α-Syn in a biosensor cell line. We show that conformationally distinct α-Syn polymorphs exhibit characteristic fluorescence lifetimes. FLIM further revealed that α-Syn polymorphs were differentially processed by not only the HSP70 disaggregation system, but also by other major cellular clearance pathways, such as the ubiquitin-proteasome system or autophagy, yielding fibrillar species with increased seeding capacity.

Conclusions:

Overall, our results suggest that incomplete cellular processing of amyloid aggregates may accelerate their prion-like propagation rather than lead to their removal.

Funded by:
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Theme	(X)
Neuropathology of prion disease	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	X
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Sporadic CJD subtype differences in human cerebral organoid infections

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#equal contribution
*presenting author

Aims: The propensity of different sporadic Creutzfeldt Jacob Disease (sCJD) subtype prions to attack different brain cell types and regions has traditionally been difficult to investigate in cell culture models of infection. We aimed to use a human cerebral organoid model of sCJD infection to investigate the manifestations of different subtypes in a fully human model of the cerebral cortex.

Materials and methods: We infected organoids heterozygous for the polymorphism at codon 129 (129MV) with four MV1 and five MV2 sCJD brains over three batches (differentiations) from the same donor induced pluripotent cell line. Organoids were harvested at various times post infection and examined for the standard parameters of prion infection, cell death, neuroelectrophysiological dysfunction, neurotransmitter receptor changes, and signal transduction pathways.

Results: In similarity with our previously reported results, the MV2 infections deposited substantially more protease-resistant PrP. No significant changes in viability were observed for the duration of the infections and some infections showed significantly decreased gene expression of pro-apoptotic intermediates. Substantial neuroelectrophysiological dysfunction was observed, beginning earlier in MV1 infected organoids than MV2. This was associated with more up-regulated neurotransmitter receptor genes in the MV1 infections than the MV2. Significant changes were observed within GABAergic, glutamatergic and serotonergic receptors. Other factors that may influence neuroelectrophysiological function were also changed without demonstration of a subtype-specific preference. These included mitochondrial gene expression and neurofilament-L gene and protein detection. Classical signal transduction pathways were not significantly changed by sCJD infection.

Conclusions: Human cerebral organoids infected with sCJD subtypes show greater prion accumulation and deposition when infected with MV2 prions but more rapid neuroelectrophysiological decline when infected with MV1 prions.

Funded by: the intramural program of the National Institutes of Health (NIAID) and grateful receipt of a CJD Foundation memorial grant.

Theme	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	X
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Infectious prion aggregates contain protease sensitive PrP that is protected from cellular degradation in a prion strain specific manner: implications for prion aggregate composition and structure

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Aims: Interactions between the cell and disease associated prion protein aggregates (PrP^D) are a critical part of prion biology, but how PrP^D changes structurally during cellular uptake and degradation remains poorly understood. We have found that, within prion aggregates, PrP^D with an intact N-terminus is present at a constant ratio to N-terminally truncated PrP^D, suggesting that it is in a part of the aggregate structure that may be protected from degradation. We therefore hypothesized that, following cellular uptake, we could monitor changes in PrP^D aggregate structure by determining the accessibility of the protected PrP^D N-terminus to proteases.

Materials and Methods: We used a combination of protease treatment, sucrose gradient centrifugation, stability in guanidinium, and immunoblotting with various antibodies to track the protease sensitivity of the N-terminus of a range of PrP^D aggregate sizes before and after cellular uptake of two murine prion strains, 22L and 87V.

Results: For both strains, PrP^D aggregates were less stable following cellular uptake. Across most aggregate sizes, increased accessibility of the N-terminus to proteases indicated significant structural change. However, within a limited size range of aggregates, the PrP^D N-terminus was preserved. This effect was strain dependent, with the N-terminus of 22L-derived PrP^D more protected than that of 87V, suggesting strain specific differences in quaternary aggregate structure. Interestingly, cell induced changes in PrP^D aggregate structure were associated with only minimal changes to the protease resistant core of PrP^D.

Conclusions: PrP^D aggregates have size-dependent differences in quaternary structure, with some potentially having a more compact structure that protects the N-terminus of PrP^D. Cells can destabilize the aggregate quaternary structure protecting PrP^D from proteases in a strain dependent manner, with structural changes that expose the N-terminus having little effect on the protease resistant core, and thus conformation, of aggregated PrP^D.

Funded by: Division of Intramural Research, National Institute of Allergies and Infectious Diseases, National Institutes of Health

Grant Number: AI000752-27

Theme: Protein structure, function, conversion, and dysfunction

Title: Comparing the Distribution of Ovine Classical Scrapie and Sporadic Creutzfeldt-Jakob Disease in Italy: Spatial and Temporal Associations (2002-2014)

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Aim: This study aims to investigate potential spatial and temporal associations between Creutzfeldt-Jakob disease (CJD) in humans (2010-2014) and ovine classical scrapie (CS) (2002-2006) in Italy, serving as a proxy for exposure.

Materials and Methods: National data from prion disease surveillance in humans (sporadic CJD) and small ruminants (CS) in Italy were utilized. A descriptive geographic analysis was conducted for each disease individually. Subsequently, an ecological study was performed to compare the occurrence of both diseases at the district and regional levels. Standardized incidence ratios (SIR), adjusted for confounders, were calculated for CJD and CS by district and region, respectively, representing the outcome and proxy of exposure. Considering a possible long incubation period of CJD, two study periods were analysed: 2010-2014 for CJD and 2002-2006 for CS. Eight alternative linear regression models were developed using SIR in humans as the dependent variable and SIR in sheep as the independent variable. These models varied in the scale of SIR data (continuous vs. categorical), geographical level (district vs. region), and the potential past exposure of sheep in specific areas to a known source of infection (via a contaminated vaccine).

Results: The analysis of data at the district level revealed no significant association. However, when considering aggregated regional data, all four models consistently indicated a statistically significant positive association, suggesting a higher incidence of the disease in humans as the regional incidence of sheep scrapie increased.

Conclusions: While the results are intriguing, it is important to acknowledge the inherent limitations of ecological studies. Nevertheless, these findings provide valuable evidence to formulate a hypothesis regarding the zoonotic potential of classical scrapie. Further investigations are necessary, employing specific designs such as analytical epidemiology studies, to test this hypothesis effectively.

Funded by: Italian Ministry of Health

Grant number: Realizzazione del programma epidemiologico finalizzato a dare evidenza del potenziale zoonotico delle TSE animali diverse dalla BSE. Prot. N. 0018730-17/07/2015-DGSAF-COD_UO-P

Acknowledgement:
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Theme
Pathogenic mechanisms in prion and prion-like diseases

THE CONTRIBUTION OF NEUTROPHIL EXTRACELLULAR TRAPS (NET) TO THE FORMATION OF TOXIC AND DIFFUSIBLE OLIGOMERS GENERATED BY AMYLOID FIBRIL DIGESTION

Ariele Martins; Thyago Cardim Pires; Fernanda Guimarães; Ana Carolina de Arantes; Patrícia Martins and **Debora Foguel**

Aims: Neutrophils are the first immune cells to arrive at the site of an infection. There, neutrophils can release NET, consisting of a cable of DNA decorated with peptides, histones, proteases etc. Our group showed that neutrophils, when incubated with amyloid fibrils (AF) in vitro, release NET in a NADPH oxidase (NOX-2)-dependent manner. Elastase associated with NET digests AF forming toxic oligomeric species. Now, these studies were extended to mice (WT and NOX2 deficient mice – KO mice), by the instillation of AF into the lungs to evaluate the contribution of NET to AF digestion and spreading. **Material and Method**: AF composed of α-Syn was instilled into the lung of WT and KO mice. The arrival of neutrophil and NET formation was quantified by immune histochemistry. Lung function was evaluated by methacholine. **Results**: After 3h of instillation of α-Syn fibrils, there is a progressive recruitment of neutrophils to the lungs of the WT and KO mice causing an extensive inflammation. AF disappeared along time in the WT lungs remaining intact in the KO lungs suggesting their fragmentation. Slices of the lung tissues of both animals revealed the presence of NET only in the WT animals, consistent with the inactivity of NOX-2 in KO mice. Furthermore, the presence of AF altered the lung function of the WT animals. Small oligomers derived from the digestion of AF were observed only in the lungs of WT mice, suggesting that the proteases present in NET might be responsible for AF degradation and oligomers formation.
Conclusions: These studies open new avenues for the participation of NET to the generation of toxic, diffusible oligomeric species that could act as prion element.
Funded by: CNPq, FAPERJ and CAPES.

Properties of emergent prion strains in Swedish moose demonstrate remarkable diversity of Nordic CWD

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Aims: With the recent emergence of CWD in Norway, Sweden and Finland, efforts to determine the potential origin are of high priority. In prior studies of Norwegian and Finnish CWD cases, we found CWD from Nordic cervids to be a sporadic, unstable form of CWD distinct from North American CWD, posing an unpredictable threat to sympatric wildlife and potentially humans. CWD recently found in Swedish moose can offer further insight into the diversity of strains that comprise Nordic CWD. Based on previous studies in Norwegian and Finnish moose CWD, we hypothesize that Sweden CWD will exhibit remarkable diversity in its transmission kinetics, biochemical properties and neuropathology when transmitted to gene-targeted mice expressing the cervid prion protein.

Materials and Methods: To characterize Swedish moose CWD isolates, we performed transmission studies on the four reported moose cases in our gene targeted (Gt) mouse models, which express cervid PrP at physiological levels. We analyzed kinetics of disease, PrP^{Sc} accumulation in the brain, histopathology, prion conformational stability and in vitro amplification by protein misfolding cyclic amplification (PMCA).

Results: We found that Swedish moose CWD isolates have diverse transmission kinetics, with disease onset of one isolate occurring after only 90 days. Conformational stability and histological features were also diverse among the Swedish isolates and differed from Norwegian and North American CWD. We found also diverse PMCA amplification profiles that suggest the presence of strain mixtures in the natural isolates.

Conclusions: Swedish moose CWD cases exhibit remarkable diversity in their properties suggesting that natural CWD isolates are comprised of strain mixtures. Future studies will determine additional properties of Swedish CWD that can increase our understanding of the mechanisms of evolution of prion diseases in the wild and their zoonotic potential.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	X
Animal prion diseases	X
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Investigating infectivity associated with prion protein deposits detected in formalin-fixed paraffin-embedded specimens from asymptomatic carriers (UK Appendix studies): an initial update.

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Aim: Current estimates for the prevalence of asymptomatic variant Creutzfeldt-Jakob disease (vCJD) infection in the UK, are based on three tissue-based studies (Appendix study I, II and III) using immunohistochemistry to detect abnormal prion protein (PrP) accumulation in archived appendectomy samples. However, there remains some uncertainty over the nature and origin of the PrP misfolded aggregates. We have recently demonstrated that abnormal PrP, extracted from vCJD formalin-fixed paraffin-embedded (FFPE) appendix tissue, and propagated *in vitro* using the highly sensitive Protein Misfolding Cyclic Amplification assay (hsPMCA), is infectious in wild-type (RIII) mice. Crucially, the material produced *in vitro* showed conservation of the vCJD strain signature. This provides a solid platform for the analysis of FFPE appendectomy samples derived from the Appendix studies. In this work, we aim to investigate the infectivity properties detected in archived FFPE appendectomy samples derived from the Appendix II and III studies.

Materials and Methods: RIII mice were experimentally challenged with inocula prepared from hsPMCA amplified PrP^{Sc}, extracted from FFPE tissue sections from five positive appendectomy specimens originating from Appendix II and III studies. A full characterisation of the clinical and histopathological features of the mice was carried out to assess infectivity and the prion strain properties of the amplified material.

Results: Interim results confirm the successful transmission to RIII mice, with clinical signs, incubation period and histopathological features consistent with the vCJD strain signature in RIII mice.

Conclusions: Our preliminary observations provide strong evidence that the abnormal prion protein deposits detected in appendectomy samples from the Appendix studies are associated with the vCJD prion agent. A complete analysis of the pathology and biochemical analyses in the recipient mice will provide a clearer understanding of the nature of the prion protein deposits found in asymptomatic carriers.

Funded by: The Policy Research Programme, Department of Health and Social Care and the Scottish Government [The National CJD Research and Surveillance Unit (NCJDRSU), PR-ST-0614-00008_18 and

Assessing and defining pre-clinical vCJD infectivity using transmission and protein aggregation models, PR-R17-0916-23001].

Theme: Pathogenic mechanisms in prion and prion-like diseases

A purely thermodynamic anti-prionic mode of action for protein-misfolding diseases

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Aims: Neurodegenerative protein-misfolding diseases, are driven by prion-like self-replicating and propagating protein assemblies of Aβ, α-synuclein, and many more. The conformation of the aggregated proteins is thermodynamically more stable than their physiological monomer conformation, which is often intrinsically disordered. Therefore, we have developed all-D-enantiomeric peptide ligands that bind the monomeric protein of interest with high affinity, thereby stabilizing the physiological intrinsically disordered monomer structure by the free binding energy. These ligands are eliminating already existing aggregates by disassembling them into monomers. This purely thermodynamic driven mode of action is truly “anti-prionic”, because it is eliminating already existing oligomers and fibrils, thus disrupting prion-like replication and propagation of toxic protein aggregates.

Materials and Methods: Atomic force microscopy (AFM), dynamic light scattering (DLS), surface plasmon resonance (SPR), nuclear magnetic resonance (NMR) and clinical trials.

Results: The all-D-enantiomeric ligand for α-synuclein, SVD-1a, disassembled preformed α-synuclein fibrils (PFF) as shown by AFM and DLS. SPR and NMR demonstrated picomolar affinity of SVD-1a to α-synuclein monomers, while keeping α-synuclein monomers in their physiological IDP conformation, which is essential for their cellular function. The all-D-enantiomeric ligand for Aβ, RD2, demonstrated ex vivo target engagement and disassembled Aβ oligomers obtained from brain tissue of former AD patients. The observed kinetic of this oligomer disassembly reaction resembles that of chaperone, indicating that RD2 acts catalytically. A clinical phase Ib, double-bind, placebo-controlled study with mild cognitively impaired (MCI) due to AD and mild AD patients treated once daily orally with RD2 or placebo for 4 weeks has been finished. The results of this study are valuable information for the scheduled phase II study.

Conclusions: The unique anti-prionic mode of action for the treatment of AD, PD and other protein misfolding diseases is promising.

Funded by: Michael-J-Fox-Foundation (MJFF), Berlin Institute if Health (BIH), and the German Federal Agency for Disruptive Innovation (Sprin-D).

Theme: Therapeutic approaches for prion and prion-like diseases

Prion 2023

Abstract Title: RNA editing alterations affect immunoregulation during sporadic Creutzfeldt–Jakob disease pathogenesis

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Aims: We aim to investigate RNA editing alterations in molecular mechanisms involved in sporadic Creutzfeldt–Jakob disease (sCJD) onset and progression, emphasizing on microglial populations. We hypothesize that altered RNA editing in microglial cells introduces RNA modifications representing a novel mechanism contributing to transcriptome diversity and subsequent translational changes associated with protein misfolding. Thus, our study focused on in vivo and in silico studies in resident (microglia) and peripheral (macrophages) immune cell populations from a humanized CJD animal model, to reveal transcriptomic and RNA editome signatures that specifically relate to increasing levels of pathology, from minimal PrP^{Sc} accumulation to massive neuroinflammatory conditions.

Materials and Methods: Tg340-PRNP129MM mice infected with postmortem material from sCJD patients of the most susceptible genotype (MM1 subtype), faithfully recapitulates the molecular and pathological alterations of sCJD human disease. Tg340-infected mice and corresponding age-matched controls were sampled in 0, 60, 120 and 180 days post-infection. Microglia and peripheral macrophages were isolated using CD11b antibody-coupled microbeads. Transcriptomic and editome profiles were obtained through deep RNA sequencing.

Results: Comprehensive global microglial RNA editomes were established in microglia and peripheral macrophages at different disease stages and reduced global RNA editing was detected. Altered transcriptomic and editome profiles were identified during disease progression. Comparative analysis between microglia and peripheral macrophages revealed RNA editing perturbations in the brain that are reflected in the periphery, even in early disease stages. T cell differentiation, peripheral nervous system neuron development and differentiation emerged as the top enriched terms

associated with common editome patterns between microglia and macrophages during disease progression.

Conclusions: Our data shed light into microglial RNA editing changes occurring during sCJD progression, enabling elucidation of disease-associated microglial processes. This establishment of specific microglia signatures that peripheral macrophages mirror at pathological conditions could mark disease and may provide biomarker potential from blood samples.

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Theme	(X)
Neuropathology of prion diseases	X
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
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Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Neuroprotective effects of Anthocyanins with anti-Prion activity

Nikoletta Christoudia [1], Eirini Kanata [2], Athanasia Chatziefsthathiou [1], Spyros Pettas [1,2], Korina Karagianni [1], Mathias Schmitz [3], Inga Zerr[3], Ioannis Tsamesidis [4], Konstantinos Xanthopoulos [2], Dimitra Dafou [1*] and Theodoros Sklaviadis[2*]

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Aims:

Prion diseases are neurodegenerative diseases (NDs) affecting mammals characterized by the conversion of the normal prion protein, PrP^{C}, into the pathogenic isoform, PrP^{Sc}. They are a group of NDs and despite ongoing research, there are no effective prophylactic or therapeutic avenues. Anthocyanins are unique flavonoids with increased interest to be used as potential therapeutics against NDs.

Materials and Methods:

Anthocyanins enriched fractions isolated from grape skins using ethanol extraction, sonication and evaporation methods were tested for their anti-oxidant and anti-prion activity. Oenin and Myrtillin, two of the most common anthocyanins in grape skins were used for the assessment of bioactivity in *in vitro* models, murine neuroblastoma N2a58, the corresponding scrapie infected cell line ScN2a22L and murine microglial cell line BV2 as well as the cell free Prion amplification assay, RT-QuiC. Cell viability in the presence of increased concentrations of these compounds was estimated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Their anti-oxidant role was estimated by the expression measurement of genes linked with the anti-oxidant response using qPCR, and by the measurement of Reactive Oxygen Species (ROS). Moreover, the assessment of their neuro- and anti-prion protection was evaluated on ScN2a22L cells, by determining via Western Blot and RT-QuiC, their ability to inhibit the accumulation of PrP^{Sc}.

Results:

Anthocyanins demonstrated their effectiveness to upregulate genes linked with anti-oxidant response, when N2a58 and ScN2a22L cells were treated. Anthocyanins are able to neutralize oxidative stress, by decreasing ROS. Treatment of ScN2a22L cells resulted in a remarkable reduction in the accumulation of PrP^{Sc}, as detected by immunoblotting. This effect was validated in cell-free assays, demonstrating that anthocyanins can prevent the formation of PrP^{Sc}.

Conclusions:

The pleiotropic beneficiary effects of anthocyanins including inhibition of PrP aggregation potentiate their use against not only prion diseases but also other neurodegenerative proteinopathies, suggesting that they could become preventative and/or therapeutic agents.

Funded by:

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Grant number:

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	X

Factors to consider when assessing risk of entry of Chronic Wasting disease and spread within cervid populations in Great Britain.

Dominika Serwin, Verity Horigan, Dan Evans, Timm Konold*, Department of Epidemiological Sciences, *Department of Pathology; DEFRA Weybridge, United Kingdom

Aims: Chronic wasting disease (CWD) has not yet been detected in Great Britain (GB); previous risk assessments (RA) have addressed the risk of entry of the disease agent from Europe. Characterisations of the European cases of CWD in reindeer, moose, and red deer, indicate, the variations of cervid TSE present in Europe do not appear to be directly related to North American CWD. Where presence of PrP^{Sc} has so far only been detected in the lymphoreticular system of reindeer whilst the prion protein has remained restricted to the central nervous system of CWD cases in European moose and red deer. RA's and control strategies should not, therefore, be based only on evidence from CWD in North America.
Materials and Methods: A RA framework for the entry and exposure of CWD in GB was used to identify data gaps. Paired with a literature review to update and identify data on importation of at-risk commodities such as live animals, fomites, plants with soil etc. Different susceptibilities of cervid species in GB based on age, sex and PRNP genotypes and the abundance and distribution were also considered. We further investigated the availability of information for potential prion variants to spread between different cervid species and to cross the species barrier.
Results: We created a guiding document which includes current information and factors to inform future risk assessments for the entry, exposure, and consequence of CWD in cervid populations in GB.
Conclusions: The emergence of novel CWD strains in Europe is a potential threat to the cervid population in GB. Knowledge of different species susceptibility to prion proteins, and the abundance and distribution of said species is imperative to estimating the likelihood of pathogen entry and spread. Helping inform high-risk target groups in GB and assisting with surveillance design to detect CWD.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	X
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Prion 2023

Title: Diagnostic Journey of Patients with Creutzfeldt-Jakob Disease (CJD) in the United States: A Real-World Evidence Study

Author list: Duncan Brown[1], Emily Kutrieb[2], Montserrat Vera Llonch[1], Rob Pulido[1], Anne Smith[1], Derek Weycker[2], Ellen Dukes[2], Brian S Appleby[3-5]

Affiliations: [1]Ionis Pharmaceuticals; [2]Policy Analysis Inc. (PAI); [3]National Prion Disease Pathology Surveillance Center; [4]Case Western Reserve University; [5]University Hospitals Cleveland Medical Center

Aims: Identification of clinical symptoms leading to a diagnosis of CJD from real-world evidence is limited. A new study using a United States (US) healthcare claims database was thus undertaken to address this evidence gap.

Materials and Methods: A retrospective cohort design and the Merative MarketScan Database (01/2012-12/2020) were employed. The study population comprised adults aged ≥18 years with ≥1 inpatient diagnosis or ≥2 outpatient diagnoses (≥3 days apart) of CJD, magnetic resonance imaging of the head or lumbar puncture, and no evidence of selected neurologic conditions after the last CJD diagnosis. Patients without healthcare coverage during the 12-month pre-diagnosis period were excluded; alternative pre-diagnosis periods (spanning 24 and 36 months, respectively) were also explored. Diagnostic journey was detailed based on diagnosis codes for selected symptoms and neurologic conditions during the pre-diagnosis period.

Results: Among the 61.8 million persons in the source population from 01/2013-12/2019, 215 CJD patients qualified for inclusion in the study population. CJD patients first presented with symptoms consistent with the diagnosis 5.0 (SD=4.0) months, on average, before the initial CJD diagnosis, and 80% had ≥3 symptoms, most commonly altered mental status (82%), gait/coordination disturbance (60%), and malaise/fatigue (44%). Most patients (63%) also had ≥1 differential (neurologic) diagnosis leading to the CJD diagnosis, most commonly cerebrovascular disease (49%), peripheral vertigo (11%), and Alzheimer's disease (7%); mean duration from first differential diagnosis to initial CJD diagnosis was 2.4 (SD=3.1) months.

Conclusions: Study findings suggest that, in US clinical practice, CJD patients present with one or more clinical symptoms impacting motor, cognitive or other domains, and many are initially mis-diagnosed, prolonging the diagnostic journey. CJD should be considered in the differential diagnosis of those with rapidly progressing dementia or motor disturbance.

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Grant number: N/A

Acknowledgment: XXX

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	X

Heparin Modulates Prion Protein Liquid-Liquid Phase Separation protecting from temperature-induced aggregation

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Aims: Transmissible spongiform encephalopathies (TSEs) are rare diseases associated with the prion protein (PrP). These diseases are caused by converting cellular PrP (PrP^{C}) to scrapie PrP (PrP^{Sc}) and subsequent aggregation, but the exact mechanism is unknown. Recent studies have shown that preliminary phase separation (PS) events contribute to the aggregation process of many proteins. Therefore, the modulation of these events is crucial to promote or inhibit protein aggregation. Previous research has demonstrated that heparin (Hep) induces transient aggregation of PrPC in a pH-dependent manner. The PrP:Hep complex is resistant to RNA-induced conversion and PrP^{Sc}-seeded conversion. This study investigates if the observed effect of Hep is associated with the PS process. We also examine the impact of temperature and pH on Hep-induced PS.

Materials and Methods: Recombinant full-length mouse rPrP23-231 and truncated rPrP90-231 were used, and data were compared to evaluate the importance of the PrP N-terminal domain. In addition, we evaluated PS of different PrP concentrations in the presence of heparin using Differential Interference Contrast (DIC), Fluorescence recovery after photobleaching (FRAP), and fluorescence microscopy.

Results: At room temperature, we observed that rPrP23-231 and rPrP90-231 phase separated in the presence of Hep. When the PrP concentration was higher, the droplets were more significant. The number of droplets was more noticeable at pH 5.5 than at 7.4. The formation of rPrP90-231 droplets was only robust in higher protein concentrations and pH 5.5. Our experiments showed that the droplets were dynamic, and we observed droplet fusion, dripping, and surface-wetting events, which confirmed their liquid-liquid nature. Increasing NaCl concentrations prevented droplet formation. We confirmed the colocalization of PrP and heparin in the droplet using fluorescence confocal microscopy. The phase separation caused by heparin was reversible, forming a soluble PrP:Hep complex. At 70 °C, the high temperature induced rapid PrP aggregation. However, it led to the formation of PrP:Hep liquid droplets.

Conclusions: Heparin alone induces the phase separation of PrP without the presence of molecular crowding agents. This effect was very dependent on the interaction of Hep with the protein flexible N-terminal domain. However, interaction with the C-terminal globular domain at acid pH also induced PS. As droplets were very dynamic and PS was reversible, PrP:Hep and PrP:PrP contacts are very dynamic. As these contacts protected PrP from temperature-induced aggregation, leading to the formation of droplets, Hep sustains PrP structural stability, preventing spurious protein contacts. Understanding the precursor states of PrP misfolding pathways and the characterization of modulators of this process provides essential information for testing the efficacy of treatments that aim to stabilize the native form of PrP.

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Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	**X**
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Assessing the Role of Syntaxin-6 on Prion Initiation, Prion Propagation and Prion-Induced Neurotoxicity *in vivo*

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Aims: Syntaxin-6 (*STX6*) is an intracellular trafficking protein, predominantly involved in early endosome to *trans*-Golgi network retrograde trafficking, which was recently proposed as a risk gene for sporadic Creutzfeldt-Jakob disease (sCJD). Increases in brain *STX6* expression is the most likely molecular mechanism driving disease risk. At Prion 2022, we presented the results of a conventional prion transmission study in *Stx6*$^{-/-}$ and *Stx6*$^{+/+}$ mice infected with RML and ME7 prions. The main aims of this work were to assess the effect of syntaxin-6 knockout *in vivo* on the establishment of prion infection, prion infectivity and prion-induced neurotoxicity.

Materials and Methods: *Stx6*$^{-/-}$ and *Stx6*$^{+/+}$ C57BL/6N mice were intracerebrally inoculated with RML prions at limited dilutions with mice culled following definite scrapie sick diagnosis and incubation period calculated. Additionally, groups of 5-10 *Stx6*$^{-/-}$ and *Stx6*$^{+/+}$ mice infected with 1% (w/v) RML prions were culled at multiple time points across the incubation period. Prion infectivity was assayed using SCEPA and the automated SCA. Age-matched neuropathology (H&E, GFAP, PrP, Iba1) was performed as well as serum neurofilament light (NfL) biomarker assessment.

Results: These findings assess the effect of syntaxin-6 knockout *in vivo* on prion initiation and the two-phase kinetics model of prion replication proposed by the MRC Prion Unit. Specifically, this work probes for differences in susceptibility to infection by performing a limiting dilution titration of RML in *Stx6*$^{-/-}$ and *Stx6*$^{+/+}$ mice. Furthermore, we determine the impact of syntaxin-6 knockout on the initial infectivity phase of exponential prion propagation by determining prion titres across the incubation period. Assessment of biomarkers and age-matched neuropathology across RML natural history indicates whether syntaxin-6 plays a role in the second neurotoxicity phase where prion titre plateaus and neuropathology becomes established.

Conclusions: This analysis helps inform potential pathological mechanisms in sCJD and elucidates the role of a risk gene, syntaxin-6.

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Acknowledgements: MRC Harwell for the generation of the mouse model. All the staff at our animal facility including Lucy Draper and Thomas Horan for breeding the lines; as well as

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	X
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Identification, Characterization and Development of Compounds Suppressing the Toxicity of Prion Protein Mutants

Giuseppe Manfroni[1], Valerio Bonaldo[3], Tania Massignan[3], Rolando Cannalire[1], Silvia Biggi[3], Marta Rigoli[3], Ilaria Vanni[12], Nicole Innocenti[3], Giulia Maietta[3], Claudia Stincardini[3], Milica Cerovic[4], Sara Cimini[4], Saioa R. Elezgarai[3], Elisa Alberti[3], Simote T. Foliaki[5], Catharina Pleschka[6], Jesùs R. Requena[7,8], Jorge M. Charco[9,10,11], Joaquín Castilla[9,10,11], Tiziana Borsello[4], Ina Vorberg[6], Steven J. Collins[5], Romolo Nonno[12], Francesca Fallarino[2], Letizia Barreca[1], and Emiliano Biasini[3]

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Aims: Several previous reports have shown that artificial deletions or disease-associated point mutations in the central hydrophobic core of PrP generate aberrant cationic currents at the cell membrane. The binding of antibodies in the C-terminal domain of PrP has been shown to induce similar effects. Moreover, electrophysiological analyses aimed at dissecting the properties of mutant PrP-induced currents in cultured cells or primary neurons have revealed a critical role for the N-terminal tail of PrP. This region contains the binding sites for PrP^{Sc}, amyloid-β oligomers (Aβ), and other disease-associated protein aggregates. These data support a model by which a physiological activity of PrP could be subverted by mutations in the central region, binding of antibodies in the C-terminus, or interaction with pathological aggregates to unleash a neurotoxic, current-forming activity encoded within the N-terminal tail of the protein.
Materials and Methods: We have designed computational and experimental approaches to study mutant PrP-mediated toxicity, which has proven effective in replicating some of the neurotoxic effects associated with PrP mutants. By capitalizing on one of these assays, we have identified a class of compounds capable of suppressing the toxic effects of mutant PrP molecules, developed synthetic schemes and conducted multiple rounds of chemical optimization to produce a series of enhanced derivatives. The most promising compounds, as evaluated in cell-based and ex-vivo assays, were profiled in silico, in vitro and in vivo for their pharmacokinetic properties.
Results: Some of the compounds we developed suppress at low nanomolar concentrations mutant PrP currents and cytotoxicity in cell cultures, PrP-dependent detrimental effects induced by Aβ oligomers in primary neurons, and PrP^{Sc}-delivered synaptotoxicity in mouse brain slices. Notably, one compound possesses suitable pharmacokinetic parameters for in vivo use.
Conclusions: These findings establish a novel class of optimized chemical agents capable of suppressing mutant PrP-mediated toxicity, making them suitable candidates for pre-clinical in vivo trials.

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Acknowledgement: We thank the Associazione Italiana Malattie da Prioni (AIEnP) and the CJD Foundation (USA) for their generous support.
Theme: Therapeutic approaches for prion and prion-like diseases

Unforeseen decrease of full-length prion protein in macaques exposed to prion contaminated blood products

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Aims: The presence of prion infectivity in blood from patients affected by variant of Creutzfeldt-Jakob disease (v-CJD) questions the risk of its inter-human transmission through transfusion. We have previously described that several cynomolgus macaques experimentally exposed to prion-contaminated blood products developed c-BSE/v-CJD; however, after an exposure to low infectious doses, the vast majority of them developed an unexpected, fatal disease phenotype focused on spinal cord involvement which does not fulfill the classical diagnostic criteria of v-CJD, notably concerning the pathognomonic accumulation of abnormal prion protein. Here we aim to investigate the etiology and physiopathology of this original myelopathy.

Materials and Methods: CNS (brain and spinal cord) samples from myelopathic macaques were tested with different biochemical approaches in comparison to samples derived from either healthy animals or their counterparts exposed to different strains of prion diseases.

Results: Current conventional techniques failed to detect any accumulation of abnormal prion protein ($PrP^{v\text{-}CJD}$) in the CNS of these myelopathic animals. Conversely, in their spinal cord we observed an alteration of their physiological cellular PrP pattern: PrP was not detectable under its full-length classical expression but mainly under its physiological terminal-truncated C1 fragment.

Conclusions: We here confirm the prion origin of this original syndrome, with a very specific biochemical signature linked to changes in PrP at the level of spinal cord lesions: contrary to what is classically described in prion diseases, host PrP is here altered in a form that is abnormally sensitive to degradation by cellular catabolism. This could provide the first experimental evidence of a link between loss of function of the cellular prion protein and the onset of disease. These observations open up new horizons in the field of prion diseases, which has hitherto been limited to pathologies associated with abnormal changes in cellular PrP towards highly structured conformations, with the possibility of unsuspected prion mechanisms/origins in certain neurodegenerative disorders.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	X
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Transmission of Idiopathic human prion disease CJD MM1 to small ruminant mouse models (Tg338 and Tg501).

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Aims: About 90% of Creutzfeldt-Jakob disease cases are classified as sporadic (sCJD), that is, occur infrequently, randomly and without a known cause. It is a fatal neurodegenerative disease with an incidence of 1-1.5 cases per million per year. Epidemiological studies have been so far unable to establish a causal relationship between sCJD and prion diseases in animals.

The zoonotic potential of sheep scrapie was demonstrated in 2014 (Cassard et al., Nature Communications) through inoculation of transgenic mice overexpressing the human prion protein with scrapie isolates. The resulting prion disease was indistinguishable from that occurring after sCJD inoculation in the same model and, while these results do not demonstrate that sCJD is caused by scrapie prions, they do show that the transmission barrier between ovine and human prions is not absolute. Our aim is to further assess this zoonotic risk.

Materials and methods: we have prepared inocula from 3 sCJD cases (MM1, MV2 and VV2) and 2 VPSPr cases (MM and MV) to verify if it is possible to recover the scrapie phenotype upon inoculation in Tg338 and Tg501 ovinized mouse models. Additionally, two different inocula gCJD (E200K) and GSS (A117V) have been also included in the bioassays as controls for classical and atypical genetic human prions, respectively.

Results: No evidence of transmission was found on a first passage in Tg338 nor Tg501 ovinized mice, but on second passage, 4/10 Tg338 mice succumbed to CJDMM1 (40% attack rate after 645 dpi) and 1/12 Tg501 mice (519dpi, 10 still alive). The remaining 2nd passages are still ongoing.

Conclusions: In this poster, the neuropathological features of the resulting strain are discussed.

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Theme (X)
X Animal prion diseases

Preliminary results of a multi-year transmission study of chronic wasting disease in white-tailed deer with rare prion protein polymorphisms

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Aims: Some prion protein (prnp) polymorphisms are underrepresented in positive cases from farmed cervid depopulation data. It is unknown if this is due to decreased susceptibility to CWD or related to the low prevalence of these genotypes. The aim of this study is to determine the susceptibility of white-tailed deer (WTD) with rare prnp polymorphisms to the agent of chronic wasting disease via exposure to other infected WTD.

Materials and Methods: Three years ago, ten WTD were oronasally inoculated with 0.1g of brain homogenate from a clinically ill CWD positive deer and placed individually into one of ten rooms. Two sentinel WTD with rare prnp genotype combinations were cohoused with each inoculated deer. Six different prnp genotype combinations were enrolled in the study: QH95GS96QQ226, QH95GG96QK226, QQ95GS96QQ226, QQ95SS96QQ226, QQ95GS96QK226, and QQ95GG96KK226. Antemortem sampling (feces, saliva, nasal swabs, ear notches, and blood) is collected every three months. Rectal biopsies were collected every three months for the first year and then annually in the sentinel deer.

Results: To date, 8/10 inoculated deer have developed clinical CWD (average IP 23, range 13-33 months) and had detectable PrPSc on postmortem testing. Four sentinel WTD have been positive on rectal biopsy immunohistochemistry: QQ95GS96QK226, QQ95GS96QQ226, and QH95GS96QQ226. One sentinel WTD (QQ95GG96KK226) reached clinical endpoints for CWD and was positive on postmortem testing. This WTD was not previously positive by antemortem rectal biopsy sampling. Up-to-date results will be presented on rectal biopsies, post-mortem analyses, and survival times for each experimental group.

Conclusions: Some exposed sentinel deer rare genotypes are susceptible to the agent of CWD. Future work will determine the onset of CWD shedding in inoculated deer to estimate the incubation period in cohoused animals that develop CWD.

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Theme	(X)
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Therapeutic approaches for prion and prion-like diseases	

Extended RT-QuIC analysis of olfactory swab diagnostic accuracy in patients with prion disorders

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Aims: In previous studies we showed that RT-QuIC assay was around 90% sensitive and 100% specific in detecting prion seeds in olfactory mucosa of patients with prion disorders. This level of diagnostic accuracy was comparable to that of CSF and RT-QuIC combination analyses of both OM and CSF samples provided a 100% diagnostic specificity and sensitivity. Still, RT-QuIC accuracy in a large number of patients with prion disorders has never been evaluated. In this real-life retrospective study, we aimed to evaluate prion RT-QuIC diagnostic accuracy on olfactory mucosa and cerebrospinal fluid, when possible, of a large cohort of CJD cases and controls.

Materials and Methods: Ninety-six patients with definite, probable CJD or with genetic prion disorders and 237 patients with non-prion neurodegenerative disorders were recruited from 2018 to 2022 in Milan, Verona and Rome neurologic clinics. All patients underwent OM swabbing (FloQBrush®, Copan, Brescia), while CSF was also obtained from eighty patients with prion disorders. RT-QuIC analysis in OM and CSF was performed using full-length and/or truncated hamster PrP, at 42°C and 55°C respectively.

Results: We found that RT-QuIC was positive in 81 out of 96 OM of patients with prion disorders and 0/237 with non-prion neurological disorders resulting in a sensitivity of 84.4%, and a specificity of 100%. Among patients with prion disorders 16 patients underwent OM swabbing only, because lumbar puncture was impracticable and 14 were RT-QuIC positive. CSF was also obtained in 80 patients with prion disorders and 66 were RT-QuIC positive 82.5% sensitive. Still, when both OM and CSF samples were considered in the same patient the sensitivity of 96.3%.

Conclusions: Here we showed that RT-QuIC diagnostic accuracy in OM sampling is 84% and that olfactory swab might replace CSF analysis when lumbar puncture is unpracticable. As we previously showed, the RT-QuIC diagnostic accuracy obtained from the combination of both CSF and OM is nearly to 100%. Of course, among “the other tissues” analyzed by RT-QuIC reported in the diagnostic criteria, OM is the best candidate.

Acknowledgement: We would like to thank Santina Castriciano for donating the flocked nasal swabs (FLOQBrushTM, Copan Italia, Spa, Brescia, Italy). We deeply thank Associazione Luca Nuti Onlus and families of CJD patients for their generous donations.

Theme (X)
Neuropathology of prion diseases
Functional protein aggregation in yeast and mammalian systems
Protein structure, function, conversion, and dysfunction
Spreading of pathology in prion-like disorders
Pathogenic mechanisms in prion and prion-like diseases
Animal prion diseases
Biomarkers for prion and other neurodegenerative diseases (X)
Therapeutic approaches for prion and prion-like diseases

A comparison of conventional and amplification prion detection methodologies with animal bioassay

McNulty, EM, Nalls, AV, Pulscher L, Mathiason CK. Department of Microbiology, Immunology, and Pathology; Colorado State University, Fort Collins, USA

Aims: Bioassay remains the gold standard for the detection of prion infectivity and has provided tremendous insight to our understanding of prion pathogenesis and transmission dynamics. The conventional prion detection methods, IHC and western blot, have been heavily relied upon for their ability to detect PrPSc deposition in tissues of prion-infected hosts. In fact, IHC is considered the gold standard diagnostic test to demonstrate prion-infection. The recent introduction of assays employing amplification of prion seeds present in tissues and bodily fluids of infected hosts, sPMCA and RT-QuIC, have enhanced our ability to identify prions during the earlier phases of disease and in samples containing minute quantities of prions. This new generation of amplification assays have reported sensitivity levels rivaling that of bioassay. Here we use a variety of methodologies to perform a cross platform comparison of these assays

Materials and Methods: To assess in vitro and in vivo prion detection methodologies, CWD positive (+) deer brain tissues homogenate (CBP6) and cervidized transgenic mouse brain tissue from and endpoint dilutional bioassay of the same brain homogenate were analyzed by IHC, western blot, sPMCA, and RT-QuIC

Results: PMCA was not more sensitive in detection positivity in bioassay than regular western blot. Regular QuIC and PMCA-QuIC have nearly the same sensitivity, but both are slightly more sensitive than PMCA alone; PMCA takes a lot longer

Conclusions: The use of “new generation” amplification assays enhances prion detection sensitivity and improves test validity for the analysis of biological samples containing very low concentrations of prions. We will never replace bioassay, but RT-QuIC and sPMCA/RT-QuIC may be useful to assess prion infectivity in concert with bioassay

Funded by: P01-5301523

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Acknowledgement: We acknowledge the students who helped with the project over the years, as well as the animal care staff for their excellent dedication

Theme	
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	(X)
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Creutzfeldt-Jakob Disease in Mexico (1990-2023): Survival analysis, population characteristics and the first bioinformatics analysis database

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Aims: Create the first database of Mexican patients with Creutzfeldt-Jakob Disease (CJD) from 1990 to 2023, to describe survival and presenting clinical features.

Materials and Methods: We performed a systematic scoping review from all published reports of Mexican patients with CJD following the PRISMA methodology. The quality was evaluated through the scale of The Joanna Briggs Institute. Likewise, we included new patients from INCMNSZ and INNN centers. Nonparametric estimation of Kaplan-Meier survival analysis was performed in R.

Results: Seventy-five Mexican patients with CJD were identified between 1990 and 2023; the median of age was 54 yo (ranges 20-90 yo). The main reported manifestations were: cerebellar or visual alterations (n=57), followed by myoclonus (n=43) and extrapyramidal alterations (n=41). The Kaplan-Meier curves show no differences in mean survival by gender (≈275 days); however, those patients reporting cerebellar and visual alterations (≈275 days) have longer survival compared to those who do not (≈125 days); Likewise, when grouping the Definite or Probable diagnosis (>400 days) in comparison to those with Possible diagnosis (200 days) of CJD; and patients with Familial etiology (≈325 days) versus patients with Sporadic etiology (≈275 days) we not found statistical differences.

Conclusions: The Creutzfeldt-Jakob Mexican cases show a longer survival than reported elsewhere; however, it could be due to classification bias (in the case of Possible diagnosis). Furthermore, the lack of application of diagnostic tools (such as 14-3-3, TAU, Biopsy, PRNP gene analysis, or RT-QUIC) does not allow the determination of Definitive or Probable diagnoses, which increases the uncertainty of misdiagnosed cases. More information is available at: https://rpubs.com/MrKristarlx07/PrionMex_survival_analysis

Funded by: F.C.-L. has the support of the Consejo de Ciencia y Tecnología del Estado de Puebla, as well as the Vicerrectoría de Investigación y Estudios de Posgrado of the Benemérita Universidad Autónoma de Puebla (Autonomous University of Puebla).

Theme	(X)

Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	X
Therapeutic approaches for prion and prion-like diseases	

Recombinant Aβ amyloid fibrils seed CAA pathology in APP23 transgenic mice

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Department of Physics, Chemistry and Biology (IFM), Linköping University, Sweden

Aims: To investigate the seeding activity of recombinant Aβ amyloid fibrils in the APP23 transgenic mice

Materials and Methods: APP23 mice, Luminescence Conjugated Oligothiophenes (LCOs), Aβ specific antibody 4G8 (Aβ epitope 18-22), Confocal microscopy.

Aβ amyloid plaques and CAA (cerebral amyloid angiopathy) in the brain are pathological hallmarks of Alzheimer's disease (AD) and vascular dementia. The spreading of Aβ pathology in the brain appears to occur through the seeding mechanism, where preformed fibrils (called seed) can accelerate fibril formation by bypassing the rate-determining nucleation step. Several studies showed that AD pathology can be induced in transgenic mice by injecting Aβ-rich brain extracts (seeds) from transgenic mice and human AD brains [1]. However, studies on recombinant seeds are limited. Therefore, we investigated the seeding activity of pure recombinant Aβ fibrils of different compositions. Seeds were injected into APP23 mouse brains at 3 months and were analyzed after 6 months of incubation.

Results: We observed that recombinant seeds (fibrils from Aβ1-42, Aβ1-40, and Aβ1-40+Aβ1-42) accelerated plaque formation in terms of number of aggregates per section compared to non-inoculated transgenic control mice. In addition, all seeds induced profound CAA in young APP23 mice (9 months). Interestingly, pure Aβ1-42 seeds produced significantly more CAA and amyloid plaques than seeds containing Aβ1-40, which is surprising given that APP23 mice produce 5-fold more Aβ1-40 than Aβ1-42.

Conclusion: This study suggests that Aβ1-42 seeds are very potent in seeding CAA.

Funded by: The study was funded by the Swedish Brain Foundation (ALZ2019-0004, and ALZ2022-0004), the Swedish Research Council (2019-04405), and the Gustav V and Drottning Victorias Foundation.

Acknowledgement: We want to thank Walker Jackson for supporting mouse colonies and experiments and Peter Nilsson for LCO dyes.

References:

1. Rasmussen et al Amyloid polymorphisms constitute distinct clouds of conformational variants in different etiological subtypes of Alzheimer's disease, PNAS 2017 Doi: 10.1073/pnas.1713215114

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	X
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Prion 2023 - Description of an atypical transmissible human prion disease linked to the P39L mutation in the prion protein gene

Federico Angelo Cazzaniga[1], Giuseppe Bufano[1], Ilaria Linda Dellarole[1], Antonio Indaco[1], Emanuela Maderna[1], Marcella Catania[1], Edoardo Bistaffa[1], Luigi Celauro[2], Hasier Eraña[3], Jorge M. Charco[3], Ilaria Vannetiello[1], Joaquín Castilla[3], Giuseppe Legname[2], Michele Angelo Di Bari[4], Daniela Galimberti[5], Chiara Fenoglio[5], Giorgio G. Fumagalli[5], Elio Scarpini[5], Giorgio Giaccone[1], Fabio Moda[1]

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[5] Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, University of Milan, Milan, Italy

Aims
The aims of this work were to characterize the brain of a patient carrying the P39L mutation in the prion protein gene (PRNP) through biochemical, immunohistochemical and animal bioassay approaches. The P39L mutation is known to be a rare cause of frontotemporal dementia but our study showed that it might be responsible for an atypical form of transmissible human prion disease.

Materials and methods
Initially, the brain was subjected to neuropathological, biochemical, and seed amplification assay (SAA) analyses (RT-QuIC and PMCA). Subsequently, the brain was intracerebrally inoculated into transgenic mice expressing human PrP with methionine at position 129 of Prnp (TgMHu2M). At the terminal disease stages, the animals were sacrificed and their brains examined through neuropathological, biochemical and SAA analyses. Additionally, RT-QuIC analyses were performed on CSF sample collected from the patient at the first visit, using different PrP substrates.

Results
The neuropathological and immunohistochemical analyses of the P39L brain did not reveal the typical hallmarks of prion diseases. However, the RT-QuIC and PMCA analyses enabled the amplification of prions from multiple brain areas. Remarkably, all the inoculated animals succumbed to the disease with a survival time of 286 ± 8 days post-injection (mean ± SEM). Once again, the standard neuropathological analyses of the mouse brains failed to detect the typical prion pathology. However, PMCA and RT-QuIC analyses successfully enabled amplification of prions from all the samples.

Conclusions
Our findings suggest that the PRNP P39L mutation induces an atypical form of prion disease, despite the fact that mutation carriers clinically presenting as having FTD. The unprecedented neuropathological and biochemical characteristics of this patient may contribute to clarify the molecular mechanisms of prion protein pathological modifications.

Funded by
This work was supported by the Italian Ministry of Health (RRC) to Fabio Moda

Grant number
RCR

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valuable and periodic support to our research projects

Theme: Protein structure, function, conversion, and dysfunction

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	X
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Prion 2023

Title: Exploring BiP-Mediated Protein Dissagregation *in vitro*

Fernando Teodoro[a], Edward Avezov[b], Eduardo Pinho Melo[a]

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[b]Department of Clinical Neurosciences, UK Dementia Research Institute, University of Cambridge, Cambridge, UK.

Aims: Protein synthesis relies on intricate cellular mechanisms to ensure proper folding and prevent the accumulation of misfolded, aggregation-prone species. While cytoplasmatic molecular chaperones are known to counteract aggregate formation by resolving insoluble protein aggregates, it remained unclear whether a similar disaggregation system exists in the Endoplasmic Reticulum (ER). Immunoglobulin-binding protein (BiP), a member of the heat shock protein (Hsp70) family in the ER, is a key ATP-dependent chaperone involved in the ER quality-control system. BiP's function is closely linked to its interaction with specific members of the ER-localized DnaJ family (ERdjs), which enhance BiP's ATP-dependent substrate interactions. In this study, we aim to demonstrate the disaggregation activity of the stress-responsive ER molecular chaperone BiP through *in vitro* reconstruction of the BiP-mediated disaggregation reaction.

Material and Methods: To investigate BiP's capacity to facilitate disaggregation, we sought to recreate this process *in vitro*. Given that J-Domain proteins play a significant role in modulating HSP70/BiP activity and are essential for recruiting the chaperone to its target substrates, a HaloTag (HT)-aggr probe was fused with a minimal generic J-Domain (HT-aggr-JD), a strategy to enhance BiP ATP hydrolysis, thus mimicking BiP/co-chaperone/substrate dynamics. BiP's disaggregase activity was evaluated through gel filtration chromatography and *in vitro* HT (dis)aggregation assays.

Results: The fusion of the J-Domain with the HT-aggr probe demonstrated functionality by effectively recruiting BiP and interfering with BiP's ATP-Induced oligomerization, unlike the HT-aggr without J-Domain. Moreover, the HT-aggr-JD fusion retained its ability to form aggregates when subjected to mild heating, allowing to observe the chaperone's capacity to act as a disaggregase core. When monitoring the probe through its ligand's fluorescence, a BiP/ATP and time-dependent shift from aggregates towards smaller species was observed. In the absence of BiP or without ATP fuelling the chaperone, the aggregates remained stable and continued to grow. Similar behaviour was observed when the aggregated probe lacking the J-Domain was used as a substrate.

Conclusions: These findings demonstrate that ATP-fueled BiP, has the capacity to disassemble pre-existing protein aggregates. However, it should be noted that the *in vitro* measurements may not fully capture the accurate kinetics of this process given the challenge of representing the intricate complexity of the BiP-assisting machinery.

Funded by: National Funding by FCT – Foundation for Science and Technology, through the 2022 Call for PhD Studentships – Regular line in scientific and academic institutions (2022.09962.BD).

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	X
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Prion 2023

Detection of CWD prions in plants collected from white-tailed deer farms

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Chronic wasting disease (CWD) affects both farmed and free-ranging cervids. Transmission of CWD is thought to occur by direct animal-to-animal contact and by exposure to contaminated environmental fomites. CWD-prion seeding activity has been detected in natural and experimentally-contaminated environmental samples including mineral licks, water sources, dust, manmade surfaces, soils, and plants. Importantly, prion infectivity in some of these samples has been proven. However, whether CWD exposed plants carry prion levels relevant to sustain infectivity has not been tested. **The aim** of this study is to explore if plants collected in a CWD contaminated facility are able to spread prion.
Materials and Methods: In this study, we optimized the detection of CWD-prions in plants using the protein misfolding cyclic amplification (PMCA). We compared NaPTA pretreatment and direct spiking of the sample into the PMCA reactions. After achieving technical optimizations, we screened multiple plant specimens collected from white-tailed deer breeding facilities displaying variable CWD prevalence. Plants from a site displaying the highest CWD prevalence were tested for infectivity in meadow voles, a co-existing animal species that feed from grass plants.
Results: Our results demonstrated that CWD-prion detection in plants was optimal when samples were pre-treated with a NaPTA-based protocol. Our screening results showed positive PMCA activity for specimens collected from the farm with the highest CWD prevalence. Although meadow voles were highly susceptible to CWD-prions by intra-cranial administration, consumption of contaminated grass did not induce prion infection in these rodents.
Conclusions: Pre-PMCA treatment with NaPTA increase the detection of CWD-prions *in vitro* in plant specimens. Although the detection of CWD in naturally contaminated vegetation was possible, the amount of prion was apparently low. This was demonstrated by the lack of transmission to meadow voles exposed to these plants. These findings further contribute to understand how CWD prions interact with multiple environmental elements.

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Grant number: 1R01AI132695 and AP20VSSPRS00C143

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	X
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Creutzfeldt-Jakob Disease Cluster Investigations: A Systematic Review

Authors: Friederike Schaper[a], Richard Knight[b], Lovney Kanguru[b]

Edinburh Medical School, University of Edinburgh[a]. National CJD Research and Surveillance Unit, Western General Hospital, Edinburgh[b].

Aims: Cases of Creutzfeldt-Jakob disease (CJD), a progressive and invariably fatal neurodegenerative disease, may form temporo-spatial clusters which could provide aetiological clues. We conducted a systematic review of CJD cluster investigations to examine the evidence basis for these, analysing whether studies have previously identified significant clusters, the methodologies of these studies, and any potential causes identified.

Methods: PubMed, MEDLINE and Scopus were searched using key terms for 'cluster(s)' and 'CJD'. All forms of CJD were eligible. No geographical restrictions were applied. Articles focusing only on genetic mutations or neuropathological/genetic analysis were excluded. Two reviewers independently screened titles, abstracts, and full texts, and undertook data extraction for included papers.

Results: Searches yielded 904 citations of which 21 were included: United Kingdom (7), France (4), Spain (2), Slovakia (2), Italy (1), Japan (3) and Australia (2). Most investigations included definitive and probable cases of sporadic (8) and variant CJD (5); 79% had a higher ratio of females to males. Of these publications, 16 identified some evidence for case clustering through their investigations, using differing definitions, but only 2 had control groups (hospital or age-matched). Most clusters were identified using statistical or epidemiological analysis (interviews of relatives, medical records), or both, over varied time-periods (6-25 years). Two articles highlighted surgery/invasive procedures (sCJD) as potential risk factors for sCJD, but without conclusive evidence. One article (UK) identified prion cross-contamination of meat products from splitting cattle heads on butcher's premises, as a risk factor for vCJD transmission.

Conclusion: 21 publications on CJD clusters were found; however, few provided firm evidence for significant clusters and only one conclusively identified a risk factor for clustering (for vCJD)**.** While clusters are an important aspect of CJD public health surveillance with potential significance for aetiology, these publications provide little evidence for CJD clustering.

Funded by: N/A
Grant Number: N/A

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
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Funded by: N/A
Grant Number: N/A

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Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	X
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Recombinant AAV-delivered functional knockout of the prion gene using CRISPR-Cas technology

Claire Verkuyl*, Ari Belotserkovsky*, Sabrina Zhu, Declan Williams, Wenda Zhao, Joel Watts, and Gerold Schmitt-Ulms. Tanz Centre for Research in Neurodegenerative Diseases, Department of Laboratory Medicine and Pathobiology; University of Toronto, Toronto, Canada.

*Presenters and equal contributors to this work.

Aims: Any therapy that profoundly reduces the brain levels of the cellular prion protein (PrP^C) should be effective for the treatment of prion diseases. This project builds on the specific hypothesis that recent advances in the virus-mediated delivery of gene therapies can be harnessed for this objective.
Aim 1: Assemble an all-in-one rAAV vector that can generate a functional knockout of the prion gene.
Aim 2: Construct a second all-in-one rAAV reporter vector for monitoring prion gene-editing efficacy.
Aim 3: Generate a robust and scalable rAAV assembly pipeline based on a brain penetrant rAAV serotype.

Materials and Methods: Two rAAVs are required to undertake this work: A therapeutic all-in-one rAAV vector that codes for a prion gene-specific guide RNA and a high-fidelity small Cas endonuclease whose expression should be active in PrP^C expressing cells. A second all-in-one rAAV 'traffic light' reporter vector codes for red (mCherry) and green (mGreenLantern) fluorescent proteins, separated by a segment of the prion gene targeted by our gene therapy. The construct is designed to indicate successful transduction with mCherry expression only and to express both fluorescent proteins in cells that have undergone the desired gene edit. The assembly into brain penetrant capsids makes use of a recently reported and publicly disseminated capsid serotype.

Results: The rAAV vectors have been assembled and validated to work as intended. A robust pipeline has been implemented that makes use of affinity capture methodology for producing high titre preparations of rAAVs. Proof-of-concept prion gene editing experiments have been successfully conducted as a precursor to prion disease survival extension experiments.

Conclusions: The key components of an rAAV-delivered gene therapy that generates a functional knockout of the prion gene are in place. Future work will need to establish the relative efficacy and safety of this approach for the treatment of prion diseases.

Funded by: Canadian Institutes for Health Research (CIHR)

Grant number: 202209PJT

Acknowledgement: N/A

Proposed theme: Therapeutic approaches for prion and prion-like diseases.

Prion 2023 - Evaluating the infectivity of PMCA-generated prions from the olfactory mucosa of a patient with sporadic Creutzfeldt-Jakob disease

Giuseppe Bufano[1,2], Federico Angelo Cazzaniga[1], Marcella Catania[1], Sara Maria Silvia Portaleone[3], Arianna Ciullini[1], Ilaria Linda Dellarole[1], Chiara Maria Giulia De Luca[1], Annalisa Lombardo[1], Giovanni Felisati[3], Luigi Celauro[4], Giuseppe Legname[4], Giorgio Giaccone[1], Fabio Moda[1]

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[4] Laboratory of Prion Biology, Department of Neuroscience, Scuola Internazionale Superiore di Studi Avanzati (SISSA), Trieste, Italy

Aims

The aim of this study was to evaluate the infectivity of prions generated by PMCA from the olfactory mucosa (OM) of a patient with sporadic Creutzfeldt-Jakob disease (sCJD) who was homozygous for methionine at codon 129 of PRNP. The brain of the patient was collected for neuropathological analysis and revealed the presence of type 1 PrP^{res}. Finally, we assessed the infectivity of prions generated by PMCA from the brain homogenate (BH).

Materials and Methods

The BH of the sCJD-129MM1 patient, along with the PMCA products obtained from BH (BH_PMCA) and OM (OM_PMCA) samples, were injected into the striatum of transgenic mice expressing chimeric human/mouse PrP^{C} with methionine at the codon 129. Additionally, samples collected from a patient with Alzheimer's disease (AD, 129MM) were analyzed and used as controls. The brains of the animals were collected and divided in two halves: the left half was used for biochemical analyses while the right half for immunohistochemical analyses.

Results

The animals injected with raw sCJD-BH developed clinical signs and were sacrificed at 204 days post injection (dpi), while those injected with BH_PMCA and OM_PMCA developed clinical signs and were sacrificed at 217 and 291 dpi, respectively. None of the animals injected with AD related material developed clinical signs of prion pathology. Neuropathological analyses of sCJD-BH injected mice confirmed the presence of PrP^{res} and mild spongiform changes mainly affecting the thalamus. The other samples are currently undergoing immunohistochemical and biochemical analyses, as well as PMCA and RT-QuIC tests.

Conclusions

Prions generated by PMCA from BH and OM of a sCJD patient are infectious and it is essential to observe specific biosafety precautions when handling this material. We are investigating whether and to what extent the PMCA generated products retain the infectious properties of the sCJD-MM1 prion or whether they acquired distinct features.

Funded by
This work was supported by the Italian Ministry of Health to Fabio Moda

Grant number
RRC, EU Neurodegenerative Disease Research (JPND) ProFFIle (AC21_2/ 00032)

Acknowledgement
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Theme: Pathogenic mechanisms in prion and prion-like diseases

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	X
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Prion 2023 - **Mutations in the non-octarepeat copper binding site of the mammalian prion protein lead to spontaneous prion formation**

Juan María-Torres[1], Thanh Hoa Tran[2], Alba Marín-Moreno[1], Juan Carlos Espinosa[1], Sara Canoyra[1], Edoardo Bistaffa[3], Fabio Moda[3], Giuseppe Legname[2,4]

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Aims

Prion diseases are rare and fatal neurodegenerative disorders caused by a change in conformation of the prion protein (PrP) from the normal cellular form (PrP^{C}) to a misfolded and infectious form (PrP^{Sc}). PrP^{C} has long been known as a copper binding protein via histidine (H) residues in the octapeptide repeats (OR) and the non-OR region located in the disordered N-terminal region of the protein. Although the functional implication of copper binding to PrP^{C} is still under investigation, copper may play an important role in the conversion to the misfolded infectious form of the protein and ultimately in prion disease. In this study, we describe transgenic mice expressing mouse prion protein replacing histidine at position 95 by tyrosine (Y) (PrP H95Y) to disrupt the non-OR copper-binding site.

Materials and methods

Several transgenic (Tg) mice were generated by standard methodology substituting the histidine (H) residues with the amino acid tyrosine (Y) at position 95 in the non-OR region of PrP^{C}. The various Tg lines were evaluated for incubation times (survival times), neuropathological changes and biochemical processing of PrP. Also transmission studies were carried to several Tg lines containing mutated or wild-type PrP.

Results

Transgenic mice overexpressing approximately two-fold PrP H95Y showed clinical signs and died at about 100 days with spongiform degeneration and atypical PK-resistant PrP^{Sc} in the brain. Moreover, inoculation of brain homogenate from terminally ill mice overexpressing PrP H95Y to mice expressing low levels of PrP H95Y or overexpressing wild-type mouse PrP, *Tga20* mice, also causes lethal, spongiform encephalopathy.

Conclusions

We can conclude that the substitution of the histidine at position 95 with a tyrosine at non-OR region could promote spontaneous PrP^{C}-PrP^{Sc} conversion and induce

transmissible neurodegenerative disease in transgenic mice, inferring a critical and unique role for the non-OR copper binding site in prion disease.

Funded by
Italian Ministry of Health to Fabio Moda
Italian Ministry of University and Research (MIUR) to Giuseppe Legname

Grants no.
RRC and FIRB

Acknowledgements

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Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	X
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Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Prion 2023 - **A novel prion clearance mechanism by SERPINA3/SerpinA3n**

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[2] Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia.
[3] ELETTRA Sincrotrone Trieste S.C.p.A, Basovizza, 34149 Trieste, Italy.

Aims:
To investigate the molecular mechanisms underlying prion diseases an extensive transcriptional analysis in prion-infected cynomolgus macaques was previously conducted. Among the various molecules examined, SERPINA3 exhibited the most significant level of upregulation/overexpression.
SERPINA3, and its mouse homolog SerpinA3n, belongs to the serine protease inhibitor family of acute-phase proteins, which show a wide tissue distribution including the central nervous system.
To test SERPINA3/SerpinA3n inhibition of target protease/s involved in the clearance of prions (PrP^{Sc}) or in the degradation of the cellular prion protein (PrP^{C}) we aim at testing various paradigms to clarifying the molecular mechanisms.

Materials and Methods:
Prion-infected and non-infected mouse neuroblastoma cell lines (respectively ScN2a and N2a) were treated with both recombinant SerpinA3n protein and conditioned media from a SerpinA3n-overexpressing cell line.
In addition, recombinant SerpinA3n protein was used to produce monoclonal antibodies in SerpinA3n KO mice through hybridoma technology. The antibody clones were tested by Western Blot analyses (WB) in SerpinA3n WT and KO mouse brain regions, as well as in ScN2a and N2a cells.

Results
SerpinA3n modulates the accumulation of prions in both murine cell lines and mouse models. SerpinA3n blocks target proteases and enhances prion accumulation, thus exacerbating the production of infectious prions.
Inhibition of SerpinA3n by small molecule compounds releases the action of target proteases leading to effective clearance of prion loads.
Moreover, one selected monoclonal antibody clone was obtained (named Mo-SerA3n.14) which yielded promising results in WB.

Conclusions:
We are currently working on the characterization of Mo-SerA3n.14 monoclonal antibody through confocal microscopy analyses of various biological samples. The availability of a reliable anti-SerpinA3n antibody would facilitate a more comprehensive study of the

molecular pathways involved in SerpinA3n action and help the identification of the target proteases involved in the prion clearance mechanism *in vivo*.

Funded by
Italian Ministry of University and Research (MIUR) to Giuseppe Legname

Grants no.
FIRB

Acknowledgements

Theme	(X)
Neuropathology of prion diseases	
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Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	X
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Theme

☐ Neuropathology of prion diseases
☐ Functional protein aggregation in yeast and mammalian systems
☐ Protein structure, function, conversion, and dysfunction
☐ Spreading of pathology in prion-like disorders
☐ Pathogenic mechanisms in prion and prion-like diseases
☐ Animal prion diseases
☐ Biomarkers for prion and other neurodegenerative diseases
☒ Therapeutic approaches for prion and prion-like diseases

Title: Antisense therapies for the treatment of neurodegenerative diseases

Authors:
Hien T. Zhao[1], Candice Junge[1], Roger Lane[1], Holly B. Kordasiewicz[1], Eric E. Swayze[1]

Affiliations:
[1]Ionis Pharmaceuticals, Inc., Carlsbad, CA, USA

Aims:
To evaluate the pharmacological effect of antisense oligonucleotides in animal models and patients with neurodegenerative disease such as Alzheimer's Disease (AD)

Material and Methods:
Preclinical models of AD such as the PS19 tauopathy mouse model were treated with antisense oligonucleotides (ASOs) targeting *MAPT* to reduce its expression. Tau pathology, hippocampal volume, neuronal loss, as well as tau seeding capability were evaluated. Optimization of ASO designs lead to identification of BIIB080, the *MAPT*-targeting clinical antisense agent. CSF levels of total-tau, as well as Tau PET signal were evaluated in mild AD patients treated with BIIB080 in a randomized, double-blind, placebo-controlled, multiple ascending dose phase 1b trial with open label extension.

Results:
Preclinical data demonstrated ASO-mediated *MAPT* suppression reduced cell-to-cell spread of oligomerized tau, reversed tau pathology, and markedly reduced neuronal and cognitive impairments without associated adverse consequences. CSF total-tau was reduced in a dose-dependent manner, and suppression was sustained for at least 6 months post treatment with BIIB080 in mild AD patients, as published in Mummery et al., 2023. Tau burden as measured by Tau PET was reduced in all brain regions, with evidence of reversal, mirroring preclinical finding in PS19 mice.

Conclusions:
Suppression of disease-causing genes such as *MAPT* could be a viable therapeutic approach to patients with AD and other tauopathies. Optimization of ASOs that have long duration of effect such as BIIB080 ASO holds the promise for identification of ASOs that may be administered with extended dosing intervals and may be applicable to other neurodegenerative conditions.

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Presentation type:

☒ oral ☐ poster ☐ both

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☒ yes ☐ no

The study was conducted under appropriate ethical guidelines:

☒ yes ☐ no ☐ N/A

For authors who do not have formal ethics review committees:
The study follows the principles of the Declaration of Helsinki.☐

A unified approach for rationally designed vaccines targeting neurodegenerative diseases

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Aims: Misfolded proteins cause neurodegenerative diseases such as Alzheimer's, Parkinson's, or the prion diseases by misfolding into specific, beta-sheet rich conformations. Many structures of disease-causing, misfolded proteins have been elucidated via cryo electron microscopy and other techniques. Insights from these structures were used to rationally design vaccines targeting epitopes that are present on the surface of the misfolded proteins, but not their natively folded precursors. The efficacy of these vaccines can be tested in a variety of animal models.

Materials and Methods: Published PDB structures and molecular models were used to select discontinuous, structured surface epitopes from the disease-causing confirmations of the amyloid beta peptide, as well as the tau, alpha-synuclein, or prion protein. These epitopes were then inserted into carefully selected, innocuous scaffold proteins that adopt beta-sheet rich structures. The resulting vaccine candidates were produced in *E. coli*, purified, refolded, and subjected to rigorous quality controls to ascertain proper folding. Wild-type animals and transgenic mice were immunized using a regular prime-boost schedule.

Results: All immunized animals developed specific immune responses capable of recognizing the engineered antigens, but, more importantly, also capable of binding the misfolded proteins that were used to design the vaccines. For the latter assays we used brain samples from animals and human patients who succumbed to the disease in question. Select transgenic mouse models were then used to test the prophylactic efficacy of the vaccines. We observed significantly delayed disease onsets in disease models for Parkinson's and several prion diseases. Additional trials now also extend to Rocky Mountain elk and white-tailed deer.

Conclusions: Insights obtained from high-resolution structures and molecular models of disease-causing, misfolded proteins allowed to design vaccines using a unified, structure-based approach. Vaccinated animals developed disease-specific immune responses, and showed significant extensions in their health-spans as compared to unimmunized controls.

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Acknowledgement: We thank Xinli Tang, Brian Tancowny, Xiongyao Wang, YongLiang Wang, and Sara Amidian for their help. Moreover, we grateful to our numerous collaborators who provided samples and animal models. Lastly, we dedicate this abstract to the memory of José Miguel Flores Fernández.

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Neuropathology of prion diseases	
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Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	X

Isolation of oligomeric prions from atypical scrapie

Vanni I[1], Rathod V[2], Pirisinu L[1], Marcon S[1], Esposito E[1], Di Bari MA[1], Riccardi G[1], D'Agostino C[1], Giovannelli M[1], Igel-Egalon A[3], Agrimi U[1], Beringue V[3], Rezaei H[3], Wille H[2], Nonno R[1]

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Aims: It is known that classical scrapie purified prions contain PrP^{Sc} fibrils, which have been recently resolved at atomic resolution[1,2]. As atypical scrapie (AS) differs from classical scrapie in clinical, pathological, epidemiological and biochemical behaviours, we purified AS PrP^{Sc} to evaluate whether such differences could be associated with different structural assemblies.

Materials and Methods: Brain homogenates from an AS sheep isolate and from a pool of ovinized mice (tg338) inoculated with the same isolate have been used for the analysis. AS-tg338 pool was subjected to a purification method[3] which uses limited proteolysis with Pronase E (PE) followed by sequential centrifugations with an iodixanol cushion. A modified version of the method was also used, in which a further treatment with proteinase K (PK) was conducted after PE to obtain PrP^{Sc} (PE) and PrP^{res} (PE+PK) purified samples. For the AS-affected sheep sample, the purification method was conducted directly replacing PE with PK[4]. Purified samples were then analysed for their structural characteristics, infectivity and strain features.

Results: From the AS-tg338, a PE+PK pellet containing the expected ~8 kDa N- and C-terminally cleaved PrP^{res} and a PE pellet containing full-length PrP^{Sc} and other PrP fragments with lower molecular weight were obtained. Parallel end-point titrations in tg338 mice confirmed that both pellets were highly infectious and retained the original strain features. Finally, EM and AFM analysis of these pellets showed no presence of fibrils, but only small and indistinct, non-fibrillar PrP^{Sc} particles were detected. Comparable, but larger, oligomeric aggregates, in absence of fibrils, were also detected in the AS-affected sheep purified sample.

Conclusions: Our data suggest that atypical and classical scrapie prions have different structural arrangements, which might explain their different behaviours. Moreover, these data align with previous evidence from some human Gerstmann-Sträussler-Scheinker cases[4], supporting the notion that fibrils are not essential for prion infectivity.

1. Kraus et al., 2021
2. Manka et al., 2022
3. Wenborn et al., 2015
4. Vanni et al., 2020

Funded by: Italian Ministry of Health

Grant number: RF-2016-02364498

Theme	
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	X
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Propagation of yeast prions in a mammalian host

Annika Hornberger[1], Joana Petushi[1], Lech Kaczmarczyk[2], Emiel Michiels[3,4], Lisa Maag[1], Frederic Rousseau[3,4], Joost Schymkowitz[3,4], Joachim Degen[5], Walker Jackson[2], Ina Vorberg[1,6] (presenting)

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Aims: Transmissible spongiform encephalopathies (TSEs) are caused by prions, self-templating infectious protein aggregates composed of the host-encoded membrane-bound prion protein PrP. The disease is characterized by a long silent phase followed by a short symptomatic phase associated with PrP accumulation, astrogliosis and vacuolation. Protein misfolding and intercellular spreading are also shared by proteins associated with much more common neurodegenerative diseases, such as tau and α-synuclein. However, while mice inoculated with prions succumb to disease, animal models expressing endogenous levels of disease-associated proteins exhibit mild to moderate neurodegeneration following injection. What exactly discriminates prions from other neurodegenerative disease-associated protein aggregates is still ill-defined. It is possible that the mechanism of prion-like protein aggregate spreading and related toxicity differ for cytosolic proteins and PrP. Alternatively, cell type-/ region-specific expression of aggregation-prone proteins or selective cellular vulnerability might account for differences in pathologies.

Materials and Methods: Here we report a novel knock-in mouse model in which the prion domain NM of *Saccharomyces cerevisiae* Sup35 prion replaces the PrP coding region, resulting in cytosolic expression of Sup35 NM. Mice were injected with NM amyloid fibrils to assess prion propagation, spreading and toxicity.

Conclusions: NM mice are vital and do not spontaneously develop disease. Intracerebral injection of recombinant NM fibrils induces progressive NM prion amplification and spreading, confirming that yeast prions adapt to the mammalian cell environment. However, NM pathology markedly differs from TSE pathology, causing only mild cognitive decline after twelve months with moderate astrogliosis and neurodegeneration but no spongiform degeneration. Instead, the progression of pathology along neuroanatomical connections shares striking similarities with pathologies in wildtype mice challenged with neurodegenerative disease-associated protein aggregates. Thus, NM prion propagation fundamentally differs from mammalian prions when propagated in the same host cell environment, resembling more closely the spreading of protein misfolding in common neurodegenerative diseases.

Theme:

- Spreading of pathology in prion-like disorders
- Pathogenic mechanisms in prion and prion-like diseases

Strain typing of two atypical scrapie cases with unusual IHC characteristics.

Inigo Chomon Elosua, Katrina McCrory, Jemma Thorn, Janet Hills and John Spiropoulos

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Aims: Classical scrapie (CS) is considered the archetypical transmissible spongiform encephalopathy (TSE) and has been described since the 18th century in the UK. CS is contagious and several strains of the agent have been isolated. This multiplicity of strains is also associated with widespread phenotypic variability in the natural host.

Atypical scrapie (AS) was first identified in Norway in 1998 and it is also referred to as Nor98. However, retrospective studies identified cases dating back to 1989 indicating that it has always been present (Bruce *et al.* 2007). In contrast to CS, AS is considered to be sporadic and is attributed to a single strain.

Through the small ruminant TSE surveillance schemes two cases of atypical scrapie were identified in UK with immunohistochemical characteristics that deviate from the expected phenotype of the disease. The agent strain of these cases is currently undergoing strain typing and the ensuing phenotype will be compared with the signature of AS agents that have been isolated previously.

Materials and Methods: Western blot and immunohistochemistry were applied to the ovine tissues that were received for confirmation via the surveillance scheme. Bioassays in tg338 mice to identify the biological properties of the agent have been initiated.

Results: Bioassays from one case have been completed whilst from the other case are ongoing. Based on incubation periods the agent appears to be compatible with the known AS strain that is usually isolated from naturally occurring AS cases. Detailed analysis of the murine samples will be performed and presented at the conference.

Conclusions: The preliminary data currently available do not support the view that the isolated agent is a novel AS strain. However, further analysis and completion of strain typing from both cases is required before any firm conclusions are drawn.

Funded by: DEFRA
Grant number: SE1962

Theme: Animal prion diseases

Conformational adaptation of the [*PIN*$^+$] prion after crossing the transmission barrier created by a mutation in the evolutionarily conserved region in the non-prion domain of Rnq1

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2. **Dept. Pharmacology, University of Nevada, Reno, USA**

Goals:
The prion state can be acquired *de novo* upon a spontaneous conformational shift, or transmitted from prions formed by homologous proteins. Primary sequence differences can create transmission barriers. Causes and mechanisms for overcoming such barriers require further investigation.
Model/Method:
[*PIN*$^+$] is a yeast prion formed by the Rnq1 protein. Rnq1 encompasses a short N-terminal non-prion domain (NPD) and a C-terminal domain with four redundant Q/N-rich prion determinants (PD). In a genetic screen we identified the NPD mutation that blocks the transmission of [*PIN*$^+$] from $Rnq1_{WT}$ to $Rnq1_{mut}$ while allowing $Rnq1_{mut}$ to form a prion *de novo*.
Results/Conclusions:
$Rnq1_{mut}$ can join [PIN^+_{WT}] aggregates, but the prion state is not inherited after eliminating $Rnq1_{WT}$. Thus, NPD mutation imposes conformational limitations on PD creating a strong transmission barrier. In rare instances of [*PIN*$^+$] transmission from $Rnq1_{WT}$ to $Rnq1_{mut}$, the [PIN^+_{mut}] prions are highly unstable. Stability of [PIN^+_{mut}]s was followed for ~200 generations. In most cultures [PIN^+_{mut}] gradually stabilized leading to either stable prions or to meta-stable variants that would not stabilize any further. The multi-step conformational adaptation model is favored for [PIN^+_{mut}] stabilization. Strikingly, the transmission barrier created by $Rnq1_{mut}$ is eliminated by deleting one of the Q/N-rich determinants within the PD. The mutation also modulates the *de novo* [*PIN*$^+$] formation: when carrying a bulky C-terminal tag, $Rnq1_{mut}$ still forms [*PIN*$^+$] but $Rnq1_{WT}$ doesn't. Analysis of Rnq1 sequences from different species revealed that the mutated position is highly conserved and that Rnq1 homologs in evolutionarily distant species carry aggregation-prone Q/N-rich domains that evolved independently. Based on these findings, on positive and negative interactions of [*PIN*$^+$] with other prions and amyloids, and on the fact that [*PIN*$^+$] is the only prion found in natural yeast isolates, we hypothesize that Rnq1's function is to regulate aggregation of other proteins, and that both prion and non-prion domains are involved.

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Acknowledgement: I am grateful to Susan Liebman for allowing me to join her lab at UNR and for continuous support.

Theme: Functional protein aggregation in yeast and mammalian systems

Title: *Using Tau seeding amplification assays for the molecular subtyping of Progressive Supranuclear Palsy.*

Authors: *Ivan Martinez-Valbuena, Seojin Lee, Hidetomo Tanaka, Shelley L. Forrest, Anthony E. Lang and Gabor G. Kovacs.*
Tanz Cenre for Research in Neurodegenerative Diseases – University of Toronto. Toronto. Canada

Aims*: To enhance understanding of the molecular mechanisms underlying disease heterogeneity in Progressive supranuclear palsy (PSP)*

Material and methods: *We performed an extensive biochemical characterization of the soluble, oligomeric tau species in 20 different brain regions from a cohort of 21 deeply phenotyped patients with PSP. This was complemented by detailed clinical, neuropathological and genetic data together with tau seeding amplification assays, proteomics and spatial transcriptomics.*

Results: *Our study has exposed a striking patient-to-patient heterogeneity in the soluble oligomeric tau species across the 20 brain regions examined. Tau seeding in motor cortex from all subjects revealed a strong correlation between the amount of soluble oligomeric tau species and the tau seeding capacity, strongly implicating these species in driving the seeding activity of tau in PSP. To identify factors that contribute to differences in seeding activity, we quantified the amount of phosphorylated tau and evaluated the physical properties of tau in these diverse brain extracts using a protease-sensitivity digestion assay that differentiates protein conformations and correlated these measures with their tau seeding capacity. Finally, we have performed the first proteomic-wide profiling together with spatial transcriptomics of the motor cortex in PSP and uncovered several mechanistic pathways that differentiate patients with high seeding activity and low and low seeding activity.*

Conclusions: *Our observations strongly suggest that that distinct molecular populations of tau contribute to the observed phenotypic differences in PSP. These data support the concept that individuals with PSP may have multiple molecular drivers of an otherwise common phenotype and emphasize the need for a novel molecular classification of PSP that will underpin the future development of personalized therapeutic approaches to curtail symptom progression.*

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
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Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	X
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

CD11c Is Not Required by Microglia to Convey Neuroprotection After Prion Infection

James A. Carroll*, James F. Striebel, Chase Baune, Bruce Chesebro, and Brent Race
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Aim:
Microglia have been shown to be critical for neuroprotection during prion infection of the CNS, and their presence extends survival in mice. How microglia impart these benefits to the infected host are unknown. Previous transcriptomics and bioinformatics studies suggest that signaling through the heterodimeric integrin receptor CD11c/CD18, expressed by microglia in the brain, might be important to microglial function during prion disease. Thus, we assessed the influence of CD11c expression on prion pathogenesis.

Materials and Methods:
We intracerebrally inoculated C57BL/6 mice and CD11c$^{-/-}$ mice with RML prions and euthanized them at 80 dpi, 120 dpi, and at clinical endpoint. We assessed gliosis, prion deposition, survival, and transcriptional changes as the disease progressed.

Results:
We demonstrate that expression of *Itgax* (CD11c) and *Itgb2* (CD18) increases in the CNS in correlation with advancing prion infection. Gliosis, neuropathology, prion deposition, and disease progression in prion infected CD11c deficient mice were comparable to infected C57BL/6 mice. Additionally, both CD11c deficient and C57BL/6 prion-infected mouse cohorts had a similar consortium of inflammatory- and phagocytosis-associated genes that increased as disease progressed to clinical stages. Ingenuity Pathway Analysis of upregulated genes in infected C57BL/6 mice suggested numerous cell-surface transmembrane receptors are signaling through Spleen Tyrosine Kinase, a potential key regulator of phagocytosis and innate immune activation in the prion infected brain.

Conclusion:
The deletion of CD11c did not influence prion pathogenesis in mice and signaling through this integrin is not involved in the neuroprotection provided by microglia.

Funded by:
This research was supported by the Intramural Research Program of the NIH, National Institute of Allergy and Infectious Diseases.

Acknowledgement:
CD11c$^{-/-}$ mice were originally produced and provided by Drs. Christie Ballantyne and Huaizhu Wu. We thank Jeff Severson for animal husbandry and Tina Thomas for tissue sectioning and immunohistochemistry.

Theme	(X)
Neuropathology of prion diseases	

Functional protein aggregation in yeast and mammalian systems	
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Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

The effect of 20S proteasome enhancement on photoreceptor survival in prion disease

James F. Striebel*, Brent Race, Chase Baune, James A. Carroll, Mikael Klingeborn, Vadim Y Arshavsky and Bruce Chesebro

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Aims:

In most prion and prion-like disorders, neuronal damage occurs in both brain and retina. Previously, we found that prion-induced retinal degeneration mainly affected photoreceptor neurons and was coincident with accumulation of misfolded, disease-associated prion protein (PrPSc). Early in disease, PrPSc accumulations were detected in photoreceptor inner segments, a specialized cellular compartment central to protein synthesis and protein degradation by the proteasome. In a *Retinitis pigmentosa* model of photoreceptor degeneration, researchers determined that misfolded rhodopsin accumulation in the inner segment caused proteasomal-impairment and subsequent photoreceptor death. Informed by these studies and our confocal analysis of prion-infected retina, we hypothesized that PrPSc accumulations were causing proteasomal insufficiency and that proteasomal enhancement might prevent prion-induced photoreceptor degeneration.

Materials and Methods:

To test this hypothesis, we obtained PA28alpha over-expressing transgenic mice (PA28αOE), in which rod photoreceptors have a 40% increase in 20S proteasomal activity. PA28αOE mice were infected with 79A scrapie and photoreceptor degeneration was tracked over time by immunohistochemistry and immunofluorescence microscopy. Additionally, RT-QuIC was used to monitor PrPSc accumulation in retina.

Results:

At the disease endpoint (160dpi), there was not a significant difference in photoreceptor number in PA28αOE vs wildtype retinas. However, at disease midpoint (126dpi), the number of photoreceptors in wildtype vs PA28αOE mice was significantly different, indicating a slightly faster rate of degeneration in the wildtype mice. The accumulation of PrPSc at disease endpoint, as measured by RT-QuIC, was not significantly different in PA28αOE vs wildtype mice. Though again, near the midpoint of disease (111dpi), there was a significant difference in PrPSc accumulation.

Conclusions:

Enhancement of proteasomal capacity by overexpression of the PA28α subunit in rod photoreceptors did not rescue photoreceptors in prion disease. However, there was indication that the rates of photoreceptor degeneration and PrPSc accumulation were affected by the increased proteasomal activity in PA28αOE mice.

Funded by: This research was supported by the Intramural Research Program of the NIH, National Institute of Allergy and Infectious Diseases.

Acknowledgements: PA28alpha over-expressing transgenic mice (PA28αOE) were kindly provided by Vadim Y Arshavsky at Duke University School of Medicine, Durham, N.C., USA.

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Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Anti-chaperone Syntaxin-6 delays prion protein fibril formation and prolongs presence of toxic aggregation intermediates

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Aims: Prions replicate via the autocatalytic conversion of cellular prion protein (PrP^C) into fibrillar assemblies of misfolded PrP. While this process has been extensively studied *in vivo* and *in vitro*, non-physiological reaction conditions of fibril formation *in vitro* have precluded the identification and mechanistic analysis of cellular proteins, which may alter PrP self-assembly and prion replication. Here, we have developed a fibril formation assay for recombinant murine and human PrP (23-231) under near-native conditions (NAA) to study the effect of cellular proteins, which may be risk factors or potential therapeutic targets in prion disease, specificalyy the protein syntaxin-6, which has been implicated as a risk factor for sporadic Creutzfeldt-Jakob disease (CJD) in genetic screening.

Materials and Methods: Kinetics PrP fibril formation in NAA were followed by Thioflavin T fluorescence, electron microscopy and super-resolution fluorescence microscopy. Toxicity of aggregation intermediates was measured in primary neurons. We analysed binding of syntaxin-6 to PrP in cellular models of prion disease (PK1, CAD5) via FRET imaging and the effect of *Stx6* deletion in PMCA reactions using brain homogenate from *Stx6* -/- mice.

Results: Analysis of kinetics in NAA revealed, counterintuitively, that syntaxin-6 is a potent inhibitor of PrP fibril formation. It significantly delayed the lag phase of fibril formation at highly sub-stoichiometric molar ratios. Instead of highly ordered fibrils, in the presence of syntaxin-6 PrP formed less-ordered aggregates containing syntaxin-6. STX6 bound to PrP fibrils at specific hotspots and interacted with PrP on the membrane of RML prion infected cells. However, when assessing toxicity of different aggregation time points to primary neurons, syntaxin-6 prolonged the presence of neurotoxic PrP species.

Conclusions: These data strongly suggest that the protein can directly alter the initial phase of PrP self-assembly and, uniquely, can act as an 'anti-chaperone', which promotes toxic aggregation intermediates by inhibiting fibril formation.

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Prof. Erich Wanker, MDC-Berlin, Germany for providing E. coli clones for protein expression and the staff of the MRC Prion Unit at UCL Biological Services Facility for animal husbandry and care. We thank Dr. Georg Meisl, University of Cambridge, for helpful discussions.

Theme	
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	x
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	x
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	(x)

Update on the Transfusion Medicine Epidemiology Review (TMER) in relation to sporadic CJD

Jan Mackenzie[1], Claire Reynolds[2], Tali Yawitch[2], Autumn St John[2], Heli Harvala[2], Richard Knight[1]

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Aims:

To establish whether sCJD can be transmitted via blood components.

Materials and Methods:

Sporadic CJD (sCJD) cases are notified by NCJDRSU to NHSBT retrospectively. Donation records are sought from UK Blood Services (UKBS) and the fate of all donations determined. For cases with a history of blood transfusion, hospital and UKBS records are examined to identify donors. Identified recipients and donors are cross-checked against the NCJDRSU database to identify any matches. Death certificates are obtained for deceased donors and recipients to/from CJD cases. Medical notes are requested on individuals where dementia is listed on the death certificate.

Results:

Between 1997-2022, a total of 1161 sCJD cases had their donation status checked. Seventy-one individuals had donations issued for clinical usage and traced to 586 recipients. 371/586 recipients had died; 9 of these were reported to have died with or of dementia but these were not believed to be cases of CJD. Thirty-four individuals with sCJD had evidence of receiving a blood transfusion, of 323 reported in the same period. They had received blood components from 310 donors. Thirteen of these donors have died; one donor died with dementia, not believed to be CJD.

Conclusions:

The results of the ongoing TMER study show no evidence for transfusion associated sCJD.

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Department of Health and Social Care Policy Research Programme and Scottish Government.

Grant number:

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Acknowledgement:

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299 words

Theme	(X)
Neuropathology of prion diseases	
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Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Strain characterisation of cattle passaged CH1641.

Janet Hills, Inigo Chomon Elosua, Katrina McCrory, Jemma Thorne and John Spiropoulos

Aims: CH1641 is a strain of classical scrapie (CS) that was first isolated from a naturally occurring infection in a Cheviot sheep and has subsequently been maintained by passage in sheep and goats. CH1641 is a CS strain that shares biochemical and immunohistochemical similarities with classical BSE (C-BSE). CH1641 also transmits readily to tg110 mice, a bovinised transgenic mouse line, in contrast to other CS strains which transmit inefficiently in the same line.

To investigate the possibility that CH1641 strain may be the origin of C-BSE, bovinised and ovinised transgenic mice were inoculated with brain from CH1641 challenged cattle to characterise the biological properties of the isolated agent.

Materials and Methods: Five cattle were inoculated intracerebrally with an ovine CH1641 source. Bovinised (tg110) and ovinised (tgShpXI and tg338) mice were subsequently inoculated with rostral medulla from these five cattle. To date, bioassays from two inocula have been completed. All inoculated mice were culled at clinical end point, and are undergoing further examination using immunohistochemistry, western blotting, and lesion profiling. CH1641 ovine sources were also bioassayed in the same mouse panels.

Results: For the bovine bioassays, tg110 mice succumbed to clinical disease 168-204 days post inoculation (dpi). The incubation periods (IP) in tg338 and TgShpXI mice were 131-227 dpi and 136-184 dpi respectively. The IP in equivalent ovine bioassays in tg110, tgShpXI and tg338 mice were185-210, 148-206 and 134-183 dpi respectively. Bioassays from previously characterised C-BSE isolates in tg110 mice produce mean IP 240 dpi whilst tg338 mice succumb to disease at >500 dpi.

Conclusions: Based on incubation period analysis the passage of CH1641 in the two cattle examined has not evolved into BSE (IP <230 dpi in both tg338 and tg110 mice). Further analysis is currently ongoing to fully ascertain the disease phenotype of the cattle passaged CH1641 in mice.

Funded by: DEFRA
Grant number: SE1962

Theme: Animal prion diseases

Detection of chronic wasting disease in third eyelids from white-tailed deer by RT-QuIC

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Aims: New chronic wasting disease (CWD) cases continue to appear in the cervid populations in North America. The current gold standard for diagnosing CWD is by immunodetection methods of infectious CWD prion protein in brain or retropharyngeal lymph nodes, which are samples that require anatomical knowledge to dissect. To obtain a more accurate prevalence estimate of CWD in wild deer, it is essential to optimize diagnostic methods that can be used on readily collected samples. The aims of this study were to explore the use of third eyelid tissue for detecting CWD in white-tailed deer using real time quaking induced conversion (RT-QuIC).

Materials and methods: We tested third eyelid tissue from 124 wild white-tailed deer with known CWD status based on immunohistochemistry (IHC) results of retropharyngeal lymph node tissue, individually (n=64) at the Pennsylvania Veterinary Laboratory and in pooled samples in 10^{-2} dilution (n=60) at the Wildlife Futures Program, in combinations of 10 total samples including either 0,1,2,4,6,8 or 10 positive samples.

Results: Out of a total of 20 white-tailed deer that were IHC-positive for CWD, 19 tested positive by RT-QuIC (95% sensitivity). All white-tailed deer previously confirmed to be IHC-negative for CWD (n=44) tested negative via RT-QuIC (100% specificity). Additionally, pooled samples from an additional 30 confirmed ELISA/IHC-positive white-tailed deer with 30 confirmed ELISA-negative white-tailed deer showed that 89% of pooled samples which included third eyelid tissue from at least one confirmed ELISA/IHC-positive white-tailed deer tested positive with RT-QuIC within 45 hours. All pools which only contained third eyelids from confirmed negative deer tested negative (100% specificity).

Conclusions: Testing of third eyelid tissue individually and as pooled samples has potential to be used for CWD detection. Third eyelid tissue is easier to collect in a field setting, thus it could be used to expand surveillance of CWD by facilitating sample collections from untrained individuals.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	X
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Overexpressing CYP46A1 in prion-infected neurons reduces PrP^{Sc} propagation

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Aims: Our laboratory has previously demonstrated a decrease in the brain specific enzyme cholesterol 24-hydroxylase (CYP46A1), which converts cholesterol into 24S-hydroxycholesterol (24S-HC) to exit the brain, in in vivo and in vitro prion infection models as well as prion disease patient samples. Treatment with the anti-retroviral reverse transcriptase inhibitor efavirenz, which has been shown to activate CYP46A1 but also has other effects, decreases PrP^{Sc} propagation and prolongs disease progression in vivo. Here, we hypothesize that overexpressing CYP46A1 in prion infected neurons will decrease PrP^{Sc}.

Materials and Methods: The murine neuroblastoma cell line N2a and catecholaminergic neuronal tumor line CAD5, infected with RML or 22L prions, were transiently transfected with plasmids encoding CYP46A1 to allow for its overexpression and grown for 72 hours. Cells were then lysed and subjected to western blotting for analysis of CYP46A1 and PrP^{C}/PrP^{Sc} protein levels via Proteintek Anti-CYP46A1 and anti-PrP 4H11 antibodies respectively. Cholesterol levels in the lysate were quantified using Amplex Red Cholesterol Assay kit. Media samples were collected at the time of lysis and evaluated for 24S-HC content by 24S-HC ELISA.

Results: Western blotting demonstrated decreased PrP^{Sc} content along with increased CYP46A1 expression in the neuronal cells overexpressing CYP46A1 when compared to control cells. Analysis of cholesterol levels in lysate and 24S-HC in media showed decreased cholesterol and increased 24S-HC consistent with the overexpression of CYP46A1.

Conclusions: Our findings show that the overexpression of CYP46A1 in prion-infected neuronal cells decreases PrP^{Sc} and point to CYP46A1 and cholesterol modulation as a logical target for therapeutic development in prion disease.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
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Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	X

Extreme deformed templating induced by an infectious C-terminal core PrP amyloid

Authors:

Nuria L. Lorenzo[1], Davy Martin[2], Sonia Veiga[1], Samanta Giler[3], Yaiza B. Codeseira[1], Sanaz Sabhezei[1], Hasier Eraña[4], Maite Freire Delgado[1], Glenn Telling[5], Enric Vidal[3], Joaquín Castilla[4,6,7], Vincent Beringue[2], Human Rezaei[2], Jesús R. Requena[1]

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Aims:

PrP^{Sc} is an amyloid with a parallel in-register stack (PIRIBS) structure, whose core spans its ~90-230 sequence. However, PrP^{Sc} is not the only PrP amyloid that can propagate and accumulate in the brain, and other non-PrP^{Sc} amyloids with cores encompassing only the C-terminal or the N-terminal portion of PrP have been demonstrated to cause prion diseases in transgenic animals that overexpress PrP. Here we present evidence of infectivity of a C-terminal PrP amyloid with an unexpected associated deformed templating process that generates an N-terminal transmissible PrP amyloid.

Materials and methods:

A bacterially expressed full-length bank vole PrP (109I) was fibrillated and inoculated intracerebrally into transgenic mice expressing ~1x bank vole PrP (109I). Two subsequent passages were performed.

Results:

All mice from the three passages developed clinical signs with average incubation times of 198, 118, and 96 dpi, respectively. Mice accumulated PK-resistant PrP fragments of ~20, ~16, and ~13 kDa in their brains, recognized by the C-terminal antibody SAF84 (160-170), but not by SAF83 (126-164) and collapsing to a ~13 kDa fragment upon treatment with PNGaseF. In addition, an N-terminal ~7 kDa fragment was identified with antibody 9A2 (99-101) after sample concentration. Such fragment was not detected in control animals of the same age.

Conclusions:

These results demonstrate that a C-terminal amyloid can trigger a prion disease with a 100% attack rate in an animal model expressing physiological levels of a wild-type prion protein. The

unexpected emergence of an N-terminal PK-resistant fragment suggests an extreme deformed templating process whereby the amyloid with a C-terminal core, besides propagating, templates in parallel propagation of a second amyloid with an N-terminal core. Possible mechanistic explanations are discussed.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	X
Spreading of pathology in prion-like disorders	
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Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

A Noah's ark approach to understand the molecular basis of the *bona fide* spontaneous prion misfolding

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Aims: Our study presents a novel methodology capable of generating *bona fide* infectious prions *de novo* and provides a comprehensive analysis of protein misfolding across a vast range of prion proteins from largest collection of PrP sequences from mammalian species. Our study involved testing **more than 380 different recombinant prion proteins** and unraveling the spontaneous misfolding propensity of each one through this innovative method.

Materials and methods:

We will show the application of Protein Misfolding Shaking Amplification (PMSA) technique with a description of all necessary components for achieving efficient and reproducible genuine protein misfolding. We have selected a few emblematic misfolded recombinant PMSA products to validate the authenticity of the infectious prions generated through our methodology.

Results:
This method has successfully yielded infectious prions from an extraordinary range of species, including bat, deer, sheep, cow, mink, pig, human, dog, rabbit, and rodents, among other hundreds of species. Our research encompasses the testing of almost 400 distinct recombinant proteins, ranking the misfolding propensities of all known (and different) wild-type prion proteins and analyzing the theoretical stability of the globular isoforms in search of potential correlation with misfolding propensity.
To validate the authenticity of the infectious prions produced through our method, we carried out comprehensive inoculation experiments that confirmed the infectivity of the newly generated prions from various species.

Conclusions:

The notable efficiency of our method in replicating the spontaneous prion misfolding provides a valuable opportunity to explore important aspects of prion diseases. Our study will allow us to address fundamental questions related to infectivity determinants, transmission barriers among species or polymorphic variants, the structural influence of specific amino acids on misfolded proteins, and the potential design of proteins with dominant negative activity. The comprehensive framework we have established through this methodology and analysis will pave the way for significant progress in prion research.

Funded by:

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	(X)
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Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Stochastic misfolding drives the emergence of distinct α-synuclein strains

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[2] Institute of Biological Information Processing, Structural Biochemistry (IBI-7), Forschungszentrum Jülich, Jülich, Germany

Aims: Distinct conformational strains of α-synuclein aggregates are believed to underlie the clinical and pathological differences among synucleinopathies such as Parkinson's disease and multiple system atrophy. It has been shown that distinct strains of α-synuclein aggregates can be generated *in vitro* by polymerizing recombinant protein using different buffer conditions. However, how α-synuclein strains are formed *in vivo* remains unknown. In this study, we examined whether unique strains of α-synuclein aggregates can arise within a consistent molecular environment.

Materials and Methods: α-Synuclein pre-formed fibrils (PFFs) were generated by polymerizing either recombinant wild-type or mutant human α-synuclein in the presence or absence of sodium chloride. The conformational properties of individual PFF preparations were assessed by limited proteolysis and cryo-electron microscopy, and then seeding behavior investigated by performing propagation experiments in hemizygous M83 transgenic mice. We also examined the conformational and propagation properties of α-synuclein aggregates from the brains of spontaneously ill homozygous M83 transgenic mice.

Results: Conformational heterogeneity was found between individual preparations of PFFs generated under identical conditions. The number of polymorphs obtained was dependent on the α-synuclein sequence, with disease-causing mutations restricting the spectrum of aggregates formed. Moreover, we found that α-synuclein aggregates formed spontaneously in the brains of homozygous M83 mice were conformationally diverse and could be classified into three distinct types. Following injection of the putative recombinant and brain-derived α-synuclein strains into hemizygous M83 mice, differences in disease onset times, cerebral α-synuclein deposition patterns, and α-synuclein conformational properties were observed.

Conclusions: The conformational diversity of α-synuclein aggregates found in PFF preparations and homozygous M83 mice demonstrates that α-synuclein can spontaneously form multiple strains within an identical environment both *in vitro* and *in vivo*. Our results suggest that stochastic misfolding drives the emergence of distinct α-synuclein strains capable of initiating unique diseases.

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Theme	**(X)**
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Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

HSP10 is a generic chaperone for neurodegenerative amyloid fibril proteins

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Aims: HSP10 is a well-known co-chaperone that interacts with HSP60 to uphold mitochondrial proteostasis. However, the co-chaperone is present in high concentration outside the mitochondria, and recent findings have shown that HSP10 can interact with amyloid proteins (1). Herein, we investigated how HSP10 from different species affects the aggregation kinetics, and fibril morphology when present during aggregation of five canonical neurodegenerative amyloid proteins. We aim to determine the interaction mechanism by identifying binding sites on the chaperone and recognition motifs on amyloid fibrils.

Materials and Methods: HSP10 interactions with Aβ1-40, Aβ1-42, PrP, Tau, and α-synuclein were investigated. HSP10 from Human (HuHSP10), *Drosophila melanogaster* (DrHSP10) and *E. coli* (GroES) were used. Assays used were Thioflavin T kinetics, Transmission electron microscopy, Nuclear magnetic resonance (NMR), amyloid staining with luminescent conjugated oligothiophenes, and binding assays using labeled HSP10.

Results: HuHSP10 and DrHSP10 inhibit the *in vitro* aggregation of all the amyloid proteins tested. If HSP10 is present during the amyloid formation it also changes the fibrillar morphology of the end stage fibrils. However, the inhibition of amyloid formation appears dependent on the chaperone concentration. When the concentration of HSP10 decreases, a drastic acceleration in aggregation can be observed for several amyloid proteins investigated. HSP10 also binds preformed fibrils, and with lysine labeling of HSP10, we found that HSP10 has a strong affinity for some amyloid fibrils, but week affinity for others. NMR spectroscopy suggests that HSP10 interacts with amyloid fibrils by binding with its seven mobile loops.

Conclusions: HSP10 interacts with several different amyloid proteins. This can either lead to inhibition or acceleration of amyloid formation, solely depending on the ratio of chaperone to amyloid protein present during aggregation. Our results illustrate the importance of HSP10 and how a decrease in concentration could lead to the acceleration of amyloid formation.

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Reference: 1. HSP10 as a Chaperone for Neurodegenerative Amyloid Fibrils. Larsson JNK, Nyström S, Hammarström P. Front Neurosci. 2022 Jun 13;16:902600.

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Protein structure, function, conversion, and dysfunction	(X)
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Targeted Protein Aggregation as a proteome editing tool

Joost Schymkowitz and Frederic Rousseau
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Aims: Amyloid-like protein aggregation is a sequence specific process that can be seeded by supplying preformed aggregates to fresh monomers. Although it is generally associated with disease, functional aggregation is widespread in all kingdoms of life and aggregate are part of the human diet. Although prion-like seeding and propagation appears to be a feature of a very limited set of proteins, the first step in this process, the induced aggregation of a given protein by preformed aggregates, can be generalized to many more proteins. We propose that inducing the loss of function of a target protein via its deliberate aggregation can be used as a proteome editing tool.
Materials and Methods: We have used an extensive range of approaches, from bio-informatics, protein and peptide biophysics, analysing protein function and aggregation in cells and in mouse models.
Results: We developed a technology that employs short peptides, called Pept-Ins, that contain two repeats of the aggregation prone region of the target protein, identified by bioinformatic means, to induce its aggregation. We have successfully targeted diverse proteins from viruses, bacteria, plants, and mammalian cells and demonstrated the therapeutic potential of this technology antibacterials, antivirals and anti-tumorals, as well as its use for PET imaging and for the generation of transgenic plants.
Conclusions: Our results suggest that the concepts from the prion field can have beneficial applications elsewhere.

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Disclosure: JS and FR and scientific founders of and advisors to Aelin Therapeutics, a company that develops this platform for therapeutic use.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	x
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Animal prion diseases	

Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Surveillance for potential zoonotic transmission of chronic wasting disease among deer and elk hunters in Colorado and Wyoming

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Aims

Epidemiologic surveillance of deer and elk hunters in areas with high prevalence of chronic wasting disease (CWD) is being conducted to reveal potential zoonotic transmission.

Materials and Methods

Hunter registry data were collected for people who purchased permits for hunting deer or elk from the Colorado Department of Wildlife (CDOW) since 1995 or from the Wyoming Game and Fish Department (WGFD) since 1996. Person-years from their first hunting year were counted for each hunter, and age-adjusted analyses were used to assess if hunters have elevated prion disease incidence compared to the general US population. Prion deaths were identified through multiple cause of death data from state mortality records or the National Death Index.

Results

Among 912,499 Colorado hunters, there were 19 observed prion disease deaths compared to an expected count of 19.4 (95% confidence interval: 11-28). Among 533,987 Wyoming hunters, there were 7 prion disease deaths, compared to an expected count of 5.4 (95% CI: 1-10). Subanalyses focusing on hunters who purchased hunting licenses in areas known to be endemic to CWD did not reveal elevated prion disease mortality.

Conclusions

Excess prion disease mortality has not been identified among deer or elk hunters in Colorado or Wyoming. However, given potentially long incubation periods, changes in the natural host or agent pathogenicity, and the continued spread of CWD, maintenance of vital surveillance projects are critical for identifying, quantifying, and substantiating potential transmissibility to humans.

Funded by: No additional funding to disclose

Grant number: Not applicable

Acknowledgement: None

Theme: Animal prion diseases

Surveillance for potential zoonotic transmission of chronic wasting disease among deer and elk hunters in Colorado and Wyoming

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[3] Wyoming Department of Health, Cheyenne, WY, USA
[4] National Prion Disease Pathology Surveillance Center, Cleveland, OH, USA

Aims

Epidemiologic surveillance of deer and elk hunters in areas with high prevalence of chronic wasting disease (CWD) is being conducted to reveal potential zoonotic transmission.

Materials and Methods

Hunter registry data were collected for people who purchased permits for hunting deer or elk from the Colorado Department of Wildlife (CDOW) since 1995 or from the Wyoming Game and Fish Department (WGFD) since 1996. Person-years from their first hunting year were counted for each hunter, and age-adjusted analyses were used to assess if hunters have elevated prion disease incidence compared to the general US population. Prion deaths were identified through multiple cause of death data from state mortality records or the National Death Index.

Results

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Conclusions

Excess prion disease mortality has not been identified among deer or elk hunters in Colorado or Wyoming. However, given potentially long incubation periods, changes in the natural host or agent pathogenicity, and the continued spread of CWD, maintenance of vital surveillance projects are critical for identifying, quantifying, and substantiating potential transmissibility to humans.

Funded by: No additional funding to disclose

Grant number: Not applicable

Acknowledgement: None

Theme: Animal prion diseases

Peripherally-challenged gene-targeted mice produce strains that recapitulate the properties of natural CWD and are distinct from intracerebrally-adapted variants

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Aims: Peripheralization of prions in lymphoid tissues is a crucial event in the pathogenesis and transmission of numerous prion diseases. Customary estimates of animal and human strain properties rely on transgenic models that focus on abrogating transmission barriers by direct infection of the CNS. Using gene-targeted (Gt) mice that regulate the physiological expression of elk or deer PrP from *Prnp* control elements, we compared the properties of CWD strains resulting from intracerebral and peripheral challenges.

Materials and Methods: Gt mice were intracerebrally or peripherally challenged for multiple passages with four North American CWD isolates. Outcome measures included times to disease, end-stage prion titers, prion conformations, PrP^{Sc} properties, and neuropathological disease profiles.

Results: While incubation times and titers were consistent during the first and second intraperitoneal passages, primary intracerebral challenges produced increased titers and subsequently faster disease upon iterative passages. Peripherally- and intracerebrally-challenged mice had distinct neuropathological profiles and PrP^{Sc} distributions and morphologies. The conformational properties and sensitivities to protease digestion of peripherally-derived prions were distinct from intracerebrally-derived prions. While the conformational profiles of peripherally-derived prions were concordant with those of the originating elk and deer CWD prions, intracerebral challenges produced altered conformational variants.

Conclusions: Distinct incubation times, prion titers, and CNS disease profiles following peripheral and intracerebral transmissions reflect diverse adaptive events suggestive of strain selection. The divergent conformations of peripherally- and intracerebrally-derived prions confirm this notion. The conformational overlap between peripherally-derived and natural elk and deer CWD prions indicates that peripherally-challenged Gt mice authentically recapitulate events occurring during CWD strain selection in natural hosts. Since, by contrast, intracerebral challenges entail an adaptive event producing altered conformers, our results question the validity of this approach to measure CWD strain properties, particularly in conventional transgenic models with limited susceptibility to disease by peripheral CWD challenges.

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	X
Pathogenic mechanisms in prion and prion-like diseases	X
Animal prion diseases	X
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Strain characterization of chronic wasting disease in bovine-PrP transgenic mice

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2 Norwegian Veterinary Institute, Ås, Norway.
3 UMR Institut National de la Recherche Agronomique (INRA)/École Nationale Vétérinaire de Toulouse (ENVT), Interactions Hôtes Agents Pathogènes, Toulouse, France.
4 Canadian Food Inspection Agency, Ottawa, Canada.

Aims:
Chronic wasting disease (CWD) is an infectious prion disease that affects cervids. Various CWD prion strains have been identified in different cervid species from North America and Europe. The properties of the infectious prion strains are influenced by amino acid changes and polymorphisms in the PrP sequences of different cervid species. This study, aimed to assess the ability of a panel of CWD prion isolates from diverse cervid species from North America and Europe to infect bovine species, as well as to investigate the properties of the prion strains following the adaptation to the bovine-PrP context.

Materials and Methods:

BoPrP-Tg110 mice overexpressing the bovine-PrP sequence were inoculated by intracranial route with a panel of CWD prion isolates from both North America (two white-tailed deer and two elk) and Europe (one reindeer, one moose and one red deer).

Results:
Our results show distinct behaviours in the transmission of the CWD isolates to the BoPrP-Tg110 mouse model. Some of these isolates did not transmit even after the second passage. Those able to transmit displayed differences in terms of attack rate, survival times, biochemical properties of brain PrP^{res}, and histopathology.

Conclusions:
Altogether, these results exhibit the diversity of CWD strains present in the panel of CWD isolates and the ability of at least some CWD isolates to infect bovine species. Cattle being one of the most important farming species, this ability represents a potential threat to both animal and human health, and consequently deserves further study.

Funded by: MCIN/AEI /10.13039/501100011033 and by European Union NextGeneration EU/PRTR
Grant number: PCI2020-120680-2 ICRAD

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	

Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	(X)
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Transmission of the chronic wasting disease agent from elk to cattle after oronasal exposure

Justin Greenlee, Jifeng Bian, Zoe Lambert, Alexis Frese, and Eric Cassmann

Virus and Prion Research Unit, National Animal Disease Center, USDA-ARS, Ames, IA, USA

Aims: The purpose of this study was to determine the susceptibility of cattle to chronic wasting disease agent from elk.

Materials and Methods: Initial studies were conducted in bovinized mice using inoculum derived from elk with various genotypes at codon 132 (MM, LM, LL). Based upon attack rates, inoculum (10% w/v brain homogenate) from an LM132 elk was selected for transmission studies in cattle. At approximately 2 weeks of age, one wild type steer (EE211) and one steer with the E211K polymorphism (EK211) were fed 1 mL of brain homogenate in a quart of milk replacer while another 1 mL was instilled intranasally. The cattle were examined daily for clinical signs for the duration of the experiment. One steer is still under observation at 71 months post-inoculation (mpi).

Results: Inoculum derived from MM132 elk resulted in similar attack rates and incubation periods in mice expressing wild type or K211 bovine *PRNP*, 35% at 531 days post inoculation (dpi) and 27% at 448 dpi, respectively. Inoculum from LM132 elk had a slightly higher attack rates in mice: 45% (693 dpi) in wild type cattle PRNP and 33% (468) in K211 mice. Inoculum from LL132 elk resulted in the highest attack rate in wild type bovinized mice (53% at 625 dpi), but no K211 mice were affected at >700 days. At approximately 70 mpi, the EK211 genotype steer developed clinical signs suggestive of prion disease, depression, low head carriage, hypersalivation, and ataxia, and was necropsied. Enzyme immunoassay (IDEXX) was positive in brainstem (OD=4.00, but non-detect in retropharyngeal lymph nodes and palatine tonsil. Immunoreactivity was largely limited to the brainstem, midbrain, and cervical spinal cord with a pattern that was primarily glia-associated.

Conclusions: Cattle with the E211K polymorphism are susceptible to the CWD agent after oronasal exposure of 0.2 g of infectious material.

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Grant number: NA

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	X
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Circadian Rhythms in PrP-Associated Transgenic Mice

Kaitlyn Forrest, Erin McNulty, Joesph Westrich, and Candace Mathiason Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, Colorado, United States

Aims: This study aims to characterize the molecular and cellular circadian rhythms in transgenic mice associated with the prion protein (PrP).

Methods: Tissues from C57b/6j, FVBKO (*Prnp* knockout), and Tg(CerPrP-E226)5037$^{+/-}$ (*Prnp* over-expressing, herein referred to as 5037) mice were assessed from indicated times. Brain and spleen *Prnp* and circadian gene expression were measured by qPCR. Immune cell populations in spleens and lymph nodes of FVBKO mice were evaluated by flow cytometry (CD45,CD3,CD4,CD8a,NKp46,B220,MHC-II,F4/80,CD11b). Bone marrow was extracted and maintained in media without additives promoting differentiation. Adhered and non-adhered cells were seeded separately and after 24 hours treated with 200nM dexamethasone for 2 hours. The next day cells were collected and stained for surface markers (CD41,CD45,CD117) and intracellularly stained for proliferation marker Ki67.

Results: C57b/6j mice showed cyclic expression of core clock and clock-controlled genes, but *Prnp* expression was arrhythmic in the brain and spleen. Compared to wildtype, FVBKO mice displayed rhythmicity in the expression of one core clock gene. Other genes either did not cycle or showed dyssynchronous patterns. 5037 mice exhibited rhythmic *Prnp* in the brain and spleen and idiosyncratic expression of circadian genes. Immunophenotyping in FVBKO spleen and lymph nodes revealed rhythmic and arrhythmic lymphocytes. B220+ cells oscillated in the spleen and lymph nodes, but CD4+ and CD8+ T cells did not. NKT cells trended toward cycling in the lymph nodes but not in the spleen, while traditional NK cells demonstrated inverse trends. FVBKO bone marrow cells display rhythmic cell populations and proliferation *in-vitro*.

Conclusions: This study provides the first characterization of molecular and cellular rhythms in *Prnp*-associated transgenic mice. The altered circadian clock in 5037 mice and non-uniform maintenance of circadian networks in FVBKO mice suggest a relationship between the circadian system and native PrP.

Funded by: NIH NIAID

Grant Number: NIH NIAID R01AI156037 and NIH NIAID R01AI112956

Theme: Pathogenic mechanisms in prion and prion-like diseases

Prion 2023 abstract title: Role of membrane anchor in conformational transition of Prion Protein

Kalpshree Gogte [1], Simon Kriegler [2], Janine Kamps [1,3], Verian Bader [4], Roland Winter [2,3], Konstanze F. Winklhofer [3,4], Jörg Tatzelt [1,3]
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Aims: The aim of the study is to investigate the role of membrane anchoring in phase transition of soluble PrP to misfolded neurotoxic conformers.

Materials and Methods: We are using an *in vitro* technique to mimic membrane adherence of PrP^C in cells using, Ni-NTA containing lipid bilayer and His-tag PrP, high resolution confocal microscopy and Fluorescence recovery after photobleaching (FRAP) assay.

Results: Our group recently showed that the N-terminus of PrP is responsible and sufficient for its phase separation in solution. However, due to association of PrP with the membrane via a GPI anchor, we speculate different behaviour of PrP phase separation at the membrane. From our *in vitro* model we find that PrP rapidly forms clusters on release from the membrane.

Conclusions: PrP lacking the GPI anchor is known to cause Gerstmann-Sträussler-Scheinker Syndrome (GSS). Implying a role of membrane anchoring in conformational transition. Our observation of rapid cluster formation on release from membrane indicates that membrane anchoring prevents PrP conformational transition.

Funded by: Deutsche Forschungsgemeinschaft
Grant number: Germany's Excellence Strategy – EXC 2033 – 390677874 – RESOLV; TA 167/6-3

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	X
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Neuronal Precursor Cell therapy for sporadic CJD in a Cerebral Organoid model

Katie Williams, Simote T. Foliaki, Brent Race, Anna Smith, Tina Thomas, Bradley R. Groveman, Cathryn L. Haigh

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Aims: Cell therapies are under clinical investigation for neurodegenerative diseases, such as Parkinson's Disease, and have shown some promise in mouse models of prion diseases. Here we use human cerebral organoids (CO) to investigate how cell therapies impact sporadic Creutzfeldt-Jakob disease (sCJD) infection.

Materials and Methods: Human cerebral organoids were differentiated from induced pluripotent stem cells (iPSCs). GFP expressing iPSCs were used to differentiate neural precursor cells (NPCs). When organoids reached 5 months of age, they were infected with sCJD brain homogenate or mock-infected with normal brain homogenate (NBH). At 90dpi organoids were embedded in Matrigel either with or without the presence of the NPCs. Organoids were then monitored for the next 90 days to the conclusion of the study. Organoids were evaluated for: neuroelectrophysiology by multi-electrode arrays, neuronal changes via western blot and immunofluorescence, changes in disease associated PrP via western blot and RT-QuIC, and cell signaling alterations with Bio-Plex assays.

Results: Neural precursor cells integrated into CO and persisted throughout the remainder of the experiment. Neuronal markers were increased with NPC treatment indicating further differentiation and integration of treatment cells. Neuroelectrophysiology showed some dysfunction with sCJD infection that was restored with NPC treatment. Organoids treated with NPCs showed bolstered cell signaling intermediates. However, few of these signaling changes were influenced by sCJD infection.

Conclusions: Neural precursor therapies can induce functional changes in a human cerebral organoid model of sCJD.

Funded by: This research was supported by the Intramural Research Program of the NIH (NIAID)

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	x

Nanoparticle vaccines against chronic wasting disease (CWD) evaluated in mouse models.

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Aims: Chronic wasting disease (CWD) is a highly contagious prion disease in free-ranging and captive cervids which currently has no effective strategies for containment. The vaccination strategy pioneered by our group targets cell-surface located PrP^C using stable aggregation-prone recombinant PrP (rPrP) isoforms for overcoming self-tolerance to PrP, and for generating self-antibodies against PrP^C. This results in self-antibodies binding to PrP^C, which sterically hinder prion conversion without adverse side effects. Previous work delivered our vaccine candidate via injection in animal models, however, for effective vaccination of wildlife an oral strategy is required. Here we describe the development and characterization of peptide nanovaccines for oral prophylaxis of CWD.

Materials and Methods: Purified deer rPrP dimer (Ddi) was quantified using BCA protein assay, purity determined using Coomassie and immunoblot of polyacrylamide gels, and function tested in ELISA. Ddi was encapsulated into poly-lactic coglycolytic acid (PLGA) nanoparticles (NP) by double emulsion.

Results: Ddi can be manufactured with minimal variability between batches. Polyacrylamide gel results show Ddi at 50 kDa as the dominant protein product, with immunoblot confirming this. Serum reactivity to Ddi in ELISA verified function. Using a 50:50 ratio of lactic acid:glycolic acid, NP were ~200-300 nm in diameter based on Dynamic Light Scattering. Encapsulation efficiency of PLGA NP containing only Ddi is 55-60%, which is above the acceptable minimum. Our NP have a consistent zeta potential of nearly -20 mV, demonstrating stability in solution. The PLGA NP are stable across a range of pH.

Conclusions: Ddi can be manufactured with minimal variability, and we can encapsulate this protein in PLGA NP. We are working to co-encapsulate Ddi and CpG oligonucleotide adjuvant. We will evaluate the immune response to empty PLGA NP, PLGA NP containing Ddi, and PLGA NP with Ddi and CpG, in mice when delivered orally or subcutaneously.

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Grant number: ALLRP 571218 – 21 and 222300851

Theme: Therapeutic approaches for prion and prion-like diseases

Comparative Pre-Mortem Testing Methods for Sporadic Creutzfeldt-Jakob Disease: A Scoping Review

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Aims: To conduct an initial exploration of what is known in the existing literature regarding comparisons between methods or combinations of methods for the premortem diagnosis of sporadic Creutzfeldt-Jakob Disease (sCJD).

Materials and Methods: A pilot scoping review of four databases (PubMed, SCOPUS, CINAHL, MEDLINE Complete) limited to studies published in English between 2011 and 2021. Abstract review of 452 studies preceded full-text review, resulting in 100 studies. This pilot includes 16 studies purposefully selected to include diverse approaches.

Results: Three themes emerged: (1) Collaboration: all studies involved some form of collaboration between stakeholders (i.e., across disciplines, institutions, or nations) related to data collection, analysis, and use of tissue samples; (2) Trends associated with biomarkers and diagnostic methods: neuroimaging and surrogate biomarkers were most frequently included in studies, though sCJD-specific biomarkers emerged in the latter half of the 2011-2021 research timeline; and (3) The parameters of effectiveness of testing methods varied between studies, though sensitivity and specificity was often a key element.

Conclusions: Postmortem (autopsy) diagnosis is currently considered the gold standard in sCJD but is not ideal. Pre-mortem diagnosis has implications related to reporting, clinical trial eligibility, and care decisions. A review of existing literature comparing premortem diagnostic approaches is critical for identifying the current state of premortem diagnostics for the most frequently occurring prion disease, sCJD. This review identifies barriers, facilitators, and new directions in premortem testing, highlighting critical non-pathological aspects of testing such as collaboration, diverse perspectives on effectiveness, and influential external factors (i.e., autopsy consent). This research highlights the importance of interdisciplinary and international collaboration and the state of emerging diagnostic practices, all of which are important considerations for the development and dissemination of emerging knowledge regarding premortem sCJD diagnostics.

Funded by: N/A
Grant number: N/A
Acknowledgement: N/A

Theme	(X)
Neuropathology of prion diseases	X
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	X
Therapeutic approaches for prion and prion-like diseases	

Prion 2023 abstract

Theme: Protein structure, function, conversion, and dysfunction

Title: Distinct PrP^{Sc} quaternary structures from different brain regions of human sCJD patients.

Satish Nemani, Leonardo Cortez, Valerie Sim

Centre for Prions and Protein Folding Diseases, Neuroscience and Mental Health Institute, Department of Medicine, University of Alberta, Edmonton, Canada.

Aims: As for all prion diseases, human sporadic Creutzfeldt-Jakob disease (sCID) can present with variable phenotypes, presumably driven by distinct conformational prion strains. Currently, the method for classifying different human strains includes codon 129 polymorphism (Methionine or Valine), electrophoretic profile of proteinase K (PK)-digested disease-associated prion protein (PrP^{Sc}), and neuropathological features. While this classification can discriminate some strains, it neither captures nor explains the full diversity of phenotypes. We have previously found that PrP^{Sc} aggregate size distributions correlate with phenotype in hamster prion disease, with subpopulation analysis detecting differences in otherwise highly similar strains. We have now applied this size distribution analysis to human sCJD brains to investigate how quaternary PrP^{Sc} structure varies within brain regions and across subtypes of sCJD.

Materials and Methods: Our lab uses asymmetric-flow field-flow fractionation (AF4) to isolate prion particles from complex brain homogenates. Its adjustable fluid crossflows can separate particles with a large size range, from monomers to fibrils, in a single run. The AF4 runs in-line with a dynamic light scattering (DLS) detector that provides size information of the fractionated particles. The isolated PrP^{Sc} particles are further characterized by RT-QuIC and electrophoretic profile after PK digestion. sCJD brain regions from a variety of strain types were obtained from the CJD Surveillance System in Canada.

Results and conclusions: We report for the first time, the application of AF4 to human CJD strains. Using our AF4/DLS method and analysis of PrP^{Sc} subpopulations, we have identified strain-specific PrP^{Sc} quaternary structures from different brain regions. Compared with the current strain classification system, this technique allows a more detailed analysis of all prion particles that may contribute to strain diversity. Furthermore, while cryo-electron microscopy is identifying strain differences in PK-resistant fibrillar PrP^{Sc}, AF4 allows the isolation and characterization of the full range of assemblies, PK-resistant and sensitive.

Funded by CJD Foundation Grants in 2021 and 2022

Acknowledgement: We thank Drs Michael Coulthart, Stephanie Booth and Gerard Jansen for their assistance acquiring the CJD samples associated with the CJD Surveillance System.

A Comparative Study of the Diagnostic Value of Real Time-Quaking Induced Conversion Assay in Multi-Site Skin and Cerebrospinal Fluid for Creutzfeldt-Jakob Disease

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2 State Key Laboratory for Infectious Disease Prevention and Control, NHC Key Laboratory of Medical Virology and Viral Diseases, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China, Beijing 102206, China.

Aims: This study aims to compare the diagnostic differences between real-time-quaking induced conversion (RT-QuIC) in multi-site skin and cerebrospinal fluid (CSF) for Creutzfeldt-Jakob disease (CJD) and evaluate their diagnostic value in the early stages of the disease.
Materials and Methods: CJD patients admitted to Xuanwu Hospital of Capital Medical University between September 2021 and April 2023 were included. Skin tissue samples (3 mm2) were collected from behind the ear, upper arm, back, and thigh using a skin punch. CSF was collected when possible. The RT-QuIC assay was performed at the Prion Disease Laboratory of the Chinese Center for Disease Control and Prevention.
Results: The study included 58 CJD patients, with a male-to-female ratio of 1:0.6 and a mean age of onset at 61.8 years. Among them, 50 cases were diagnosed as sporadic CJD and 8 cases as genotypic CJD. The mean time from onset to biopsy was 5.3 months, with 35 patients biopsied within 3 months. The skin RT-QuIC had a higher overall positive rate (93.1%) compared to CSF (68.8%) ($P<0.05$). Some patients had positive skin but negative CSF RT-QuIC results, and vice versa. Skin biopsy sites showed similar positivity rates. Among patients with sporadic CJD, the skin RT-QuIC had a higher positivity rate (96.0%) compared to CSF (69.8%). For genotypic CJD, the positivity rates for skin and CSF RT-QuIC were lower but not statistically significant. Patients with an onset-to-biopsy time of less than 3 months had higher positivity rates for both skin and CSF RT-QuIC compared to those with longer intervals.
Conclusion: In Chinese CJD patients, cutaneous RT-QuIC has a higher positive rate than CSF, and simultaneous testing can be complementary. A multi-site skin biopsy may improve the diagnostic rate of skin positivity. RT-QuIC shows important diagnostic value in the early detection of CJD.

Funded by: National Natural Science Foundation of China
Grant Number: 8227146 and 81971011
Acknowledgment: None

Theme: Biomarkers for prion and other neurodegenerative diseases

Unraveling the Unique Genetic and Clinical Signatures of Prion Diseases in China: Insights from a Pioneering Single-Center Investigation

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Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing, China.

Aims: This study aimed to analyze the genetic, clinical, and ancillary examination characteristics of prion diseases in China, focusing on a single center.
Materials and Methods: Patients diagnosed with prion diseases and admitted to Xuanwu Hospital of Capital Medical University between January 2012 and July 2023 were included. Data on genetic profiles, clinical manifestations, and ancillary examinations (MRI, EEG, cerebrospinal fluid biomarkers, and RT-QuIC) were collected. Using the clinical diagnostic criteria of MM2C and MM2T types proposed in 2020 and 2018, approximate pathological speculations were made for the included sporadic Creutzfeldt Jakob disease (sCJD) patients.
Results: A total of 287 prion patients were included, with a male-to-female ratio of 1:0.9, mean age of onset of 60.1 years, and median disease duration of 1.1 years. Genetic prion diseases accounted for 13.6% of cases, with 248 sCJD, 24 genetic CJD (gCJD), 12 fatal familial insomnia (FFI), and 3 Gerstmann-Straussler-Scheinker syndrome (GSS) cases. gCJD cases included 9 E200K, 8 T188K, 2 V180, and one G114V, 7-OPRI, R148H, T193I, and V210I. FFI and GSS cases had D178N-129M and P102L genotypes, respectively. Common findings in CJD patients were high DWI signals, periodic sharp wave complexes on EEG, positive cerebrospinal fluid biomarkers (14-3-3 protein, total tau), positive skin/cerebrospinal fluid RT-QuIC, and PRNP-129M haplotype. Among 235 sCJD patients, 25.1% were probable MM2 type CJD, including 22.6% MM2C and 2.6% MM2T cases. Heidenhain's variant of CJD accounted for 8.0% and included mutations in PRNP (5 cases), T188K (3 cases), E200K (1 case), and V210I (1 case).
Conclusion: This study reveals distinct genetic distributions, clinical features, and pathological classifications of prion diseases in China compared to Caucasian populations.

Funded by: National Natural Science Foundation of China
Grant Number: 8227146 and 81971011
Acknowledgment: None

Theme: Biomarkers for prion and other neurodegenerative diseases

Enhanced CJD surveillance in the 65+ population: A Clinicopathological study of selected cognitive impairment cases in Lothian, Scotland

Authors: Lovney Kanguru[1*], Sarah Cudmore[1,] Gemma Logan[2], Briony Waddel[3], Colin Smith[1,4], Anna Molesworth[5], and Richard Knight[1]

Background: Variant Creutzfeldt-Jakob disease (vCJD) is primarily associated with dietary exposure to bovine-spongiform-encephalopathy. It may be missed, particularly in the elderly where referral to specialist neurological services might take place less often because another, more common, brain disease might be suspected. We aimed to determine the feasibility of a method to detect possible missed patients in the elderly, identify any such patients, and review the challenges encountered.

Methods: A multi-site study was set-up in Lothian in 2016. With consent, patients with 'atypical' features were referred, recruited, and clinicopathological investigations undertaken. Recruitment was however, lower than predicted, and in 2017, a review was undertaken exploring possible reasons for low recruitment using a curated-database from Anne Rowling clinic, and a questionnaire to clinicians in medicine of the elderly, psychiatry of old age and neurology specialties.

Results: 30 patients are included: 63% male, 37% female. They were referred because of at least one 'atypical' neurological feature: cerebellar ataxia, rapid progression, or somato-sensory features. Mean-age at symptom-onset (66 years, range 53–82years), the time between onset-of-symptoms and referral (7 years, range 1–13years), and duration-of-illness from onset-of-symptoms to death/censor-date (9.5 years, range 1.1–17.4 years) were determined. By 30.04.2023, 4 patients were alive, and 26 had died of-whom, 14 underwent neuropathological investigations.

The review found 25% of the patients referred were recruited. Majority had been referred because of diagnostic uncertainty. Twelve of 60 participating clinicians completed the questionnaire: high workload, time constraints, and forgetting to refer were some of the reasons given for low referral. Suggestions to improve recruitment were implemented, but without success.

Conclusion: The surveillance approach that was used was well received. No missed cases of vCJD were found. However, there remains uncertainty whether this is because missed cases are very uncommon or because the study had insufficient power to detect them.

Structural and mechanistic insights into the pathogenesis of prion diseases

Luigi Russo[1], Giulia Salzano[2], Luigi Celauro[2], Edoardo Bistaffa[3], Fabio Moda[3], Nataliia Ventserova[1], Manoj Madheswaran[1], Gianluca D'Abrosca[1], Gaetano Malgieri[1], Danilo Milardi[4], Gabriele Giachin[5], Carla Isernia[1], Giuseppe Legname[2] and Roberto Fattorusso[1].

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Aims: The main aim of this research was to describe, at atomic level, the molecular basis as well as the conformational equilibria controlling the prion misfolding process by which PrPC converts into amyloid fibril.

Materials and Methods: We used a multidisciplinary approach by combining NMR methodologies, such as melting and Relaxation Dispersion experiments, with ThT aggregation assays and RT-QuIC experiments.

Results: We provide a high-resolution description of a β-sheet-enriched intermediate state (β-PrPI) detected during the folding mechanism of the pathological human prion protein (HuPrP(90-231)). NMR structural data reveal that β-PrPI, in the regions surrounding the native two β-sheets and the α1, samples conformation with a significant tendency to increase the β-sheet folding with respect to the native state without compromising the α-helices stability. We also demonstrate that HuPrP(23-231) presents a self-regulated folding mechanism in which the N-ter domain, interacting transiently with the C-ter domain through electrostatic interactions, avoids the formation of dangerous intermediate misfolded states by tuning long-range µs-ms conformational dynamics. Finally, we show that the on-pathway β-PrPI intermediate state induces amyloid fibril formation occurring via a specific assembly mechanism involving transient oligomeric species.

Conclusions: This study provides novel structural and dynamical insights into prion misfolding that can be used to develop molecular strategies able to inhibit PrP amyloid fibrils aggregation.

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	X
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Abstract title: Strain interference in brain from FVB mice exposed to ME7 and RML prions.

Authors:

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Aims: Prion strains produce distinct phenotypes in the same host and are believed to be due to biochemically distinct disease-related PrP conformers. It is not known how interference between strains affects the kinetics of prion propagation.

Material and Methods: FVB mice were inoculated intracerebrally with ME7 and then 30, 50 or 80 days later with RML prions in equal amounts. The two prion strains were also inoculated in reverse with RML administered first and 50 days later, ME7. The mice were culled at set time points up to the point of clinical scrapie diagnosis and brains homogenised and assayed using a cell based prion infectivity assay, specific to RML prions.

Results: FVB mice showed comparable incubation periods when exposed to ME7 and RML prions respectively (in days ± SD; 182 ± 8 and 165 ± 11). Mice inoculated with ME7 and RML using intervals of 50 and 80 days, showed an incubation period in agreement with ME7 (in days ± SD; 165 ± 12 and 179 ± 7) and not RML (in days ± SD; 115 ± 12 and 99 ± 6). When brains were assayed for RML prion infectivity, the kinetics of RML prion propagation was practically unperturbed. When the two strains were inoculated in reverse, the incubation period was in agreement with RML (in days ± SD; 157 ± 6 and not ME7 (in days ± SD; 107 ± 6).

Conclusions: We see little evidence of strain interference in mice exposed to ME7 and RML prions. Incubation times suggest that the first strain inoculated takes precedence, perhaps an indication of a necessary and limiting interaction with the host. Furthermore, the prion propagation of the yielding strain, is not grossly affected, confirming that two strains can thrive in a host during the course of illness.

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X Pathogenic mechanisms in prion- and prion-like diseases

A Novel Therapeutic Approach Modulates Protein Interactions and Cellular Processes

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2. Braude College of Engineering, biotechnology engineering department, Israel
3. Colorado State University, the prion research center, USA

Aims: Recent advancements in drug development have led to the discovery of a promising therapeutic approach that effectively modulates protein interactions and influences key cellular processes. This study investigates the effects of a novel drug on various cellular activities and its potential implications for therapeutic interventions.

Materials and Methods: In vitro and in vivo cancer cell models have been used to identify the effect of the novel drug on cell survival using microscopic images of cells followed by proliferation assays. The link to PrP reduction was done using SiRNA and Western blot analysis for PrP and a normalizing protein. To identify the pathways that are involved transcriptomic analysis in combination with the metascape analysis was performed. Triple-negative cancer cell lines were used in mouse and humanized animal models.

Results: The drug downregulates Prion Protein levels in various cancer cell lines and exhibits significant efficacy in regulating immune responses, cellular function, and disease progression. The prion protein, demonstrates significant involvement in tumorigenesis and tumor progression, affecting cell survival, proliferation, and metastasis-related signaling pathways. Dysregulated prion protein expression is observed in different cancer types, highlighting its potential as a therapeutic target. Furthermore, transcriptomic analyses provide valuable insights into the molecular mechanisms underlying the drug's effects. In vivo studies using mouse and human cancer models validate the efficacy of the drug, demonstrating significant reductions in tumor growth and improved outcomes.

Conclusions: These promising results highlight the drug’s therapeutic potential in treating cancer and other diseases. This study presents a novel therapeutic approach that effectively modulates protein interactions and influences key cellular processes. The findings shed light on the potential of targeting specific extracellular proteins and their impact on immune responses, cellular function, and disease progression. Further research and development in this field hold promise for the development of innovative therapies with broad clinical applications.

Theme: Cancer

CWD in Europe: risk of introduction in Portugal?

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Aims: Chronic wasting disease (CWD), a naturally transmissible spongiform encephalopathies caused by an unconventional etiologic agent (prion), is difficult to eradicate. It is endemic in North America, and has also been diagnosed in South Korea and Scandinavia. The highly resistant prion remains in contaminated environments and transmission can occur through contaminated objects used for hunting in affected areas. The importation of cervids and their products may also contribute to spreading. Within the WastingPrionRisk project, to assess the risk of CWD entry into Portugal, the importation of cervids and the existence of hunters in Portugal from risk countries were analyzed. The risk associated with Portuguese hunters who may have gone hunting in risk countries was not considered, as no records on this activity were found.

Materials and Methods: Data on cervid imports and non-resident permits in Portugal for the 2018/2019 and 2019 /2020 hunting seasons were requested from the Competent Authorities. Online surveys were also developed and distributed to game managers and hunters, to assess the risk associated with the movement in Portugal of hunters from the risk countries: United States of America, Canada, South Korea, Norway, Finland and Sweden.

Results: On imports, 157 cervids were imported from Spain for breeding; 203 hunters from risk countries have requested authorization to hunt in Portugal. From the 184 surveys received, 48 reported the presence of foreign hunters, 8 indicating hunters from those countries.

Conclusions:
It was not possible to define risk areas; however, we conclude that there is a potential risk of CWD entering Portugal through hunters coming from risk countries. It is recommended that more complete records on the geographical area of hunting activity by non-residents hunters in Portugal should be considered, contributing to a better analysis of the risk associated with the entry and dispersion of infectious diseases in our country.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	(X)
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Detection of chronic wasting disease prions in processed meats.

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Aims: identify the presence of CWD prions in processed meats derived from elk.

Materials and Methods: In this study, we analyzed different processed meats derived from a CWD-positive (pre-clinical) free-ranging elk. Products tested included filets, sausages, boneless steaks, burgers, seasoned chili meats, and spiced meats. The presence of CWD-prions in these samples were assessed by PMCA using deer and elk substrates. The same analyses were performed in grilled and boiled meats to evaluate the resistance of the infectious agent to these procedures.

Results: Our results show positive prion detection in all the samples analyzed using deer and elk substrates. Surprisingly, cooked meats displayed increased seeding activities. This data suggests that CWD-prions are available to people even after meats are processed and cooked.

Conclusions:

These results suggest CWD prions are accessible to humans through meats, even after processing and cooking. Considering the fact that these samples were collected from already processed specimens, the availability of CWD prions to humans is probably underestimated.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	X
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Investigating the Role of Hsp110 in Prion Fragmentation and Propagation

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Aims: We hypothesize that Hsp110 participates in the disaggregation and fragmentation of PrP^{Sc} and represents a cellular modifier of prion infection. Prion diseases are caused by the misfolding of PrP^{C} into the pathological isoform PrP^{Sc}. Over time, PrP^{Sc} forms fibrillar aggregates that are fragmented/disaggregated to recruit and convert new PrP^{C}. Despite the importance of fibril fragmentation for the propagation of PrP^{Sc}, the molecular entities involved in this process are unknown. Based on increasing evidence showing that heat shock protein 110 (Hsp110) family members (Apg-1, Apg-2 and Hsp105) are part of a mammalian disaggregase machinery, we started to investigate the role of Hsp110 in cell and animal models of prion infection. Defining the mammalian machinery that disaggregates PrP^{Sc} fibrils will fill an important knowledge gap in prion biology.

Materials and Methods: Effects of transient and stable knock-out (KO) or over-expression of one or two family members combined on acute and persistent prion infection were studied in cultured cells. Mice overexpressing Hsp110 were infected with prions and analyzed for changes in PrP^{Sc} properties in the brain.

Results: Transient knockdown of Apg1 and Hsp105 shows significant reduction in PrP^{Sc} levels in N2a and CAD5 cells infected with different prion strains. N2a knockout cells lacking Apg2 or Hsp105 demonstrate reduced susceptibility to *de novo* infection with prions. Transient overexpression of Apg1 and Hsp105 showed increased levels of PrP^{Sc}. Furthermore, we observed changes in PrP^{Sc} levels and survival times in Apg1-overexpressing transgenic mice compared to wild-type controls when infected with prions.

Conclusions: Current *in vitro* evidence suggests that Hsp110 affects prion propagation likely through fragmentation as seen by increased levels of PrP^{Sc} when these proteins are overexpressed, and reduced levels when knocked down. Our studies will result in new insights into prion infection, providing a 'missing link' in the understanding of prion propagation.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	X
Spreading pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Abstract title: The mutational landscape of the Sup35 QN-rich domain reveals an essential region for Sup35 nucleation

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Aims: Despite being one of the most intensively studied prions, the mechanisms by which Sup35 nucleates transmissible aggregates from a previously soluble state are not well understood. The formation of Sup35 aggregates is driven by its N-terminal domain and small differences in its primary sequence, such as single amino acid changes, are enough to create a barrier for Sup35 nucleation. The lack of systematic characterization of these mutants hinders the understanding of the sequence and structural features that are key for Sup35 nucleation. By simultaneously quantifying the effect of hundreds of Sup35 mutants, we aim to uncover the features that govern Sup35 nucleation.

Materials and methods: Using deep mutagenesis, we built a library encompassing all single amino acid changes in the QN-rich region (aa 2-40) of the Sup35 N-terminal domain. We then employed a massively parallel approach that combines high-throughput sequencing with a selection assay using *S. cerevisiae* cells with a premature STOP codon in the adenine gene. Soluble Sup35 acts as a translation termination factor, but when nucleated it causes a STOP codon read-through and allows growth in a medium without adenine. We quantify the ability of each Sup35 variant to nucleate endogenous Sup35 by calculating the enrichment scores of the variants after growing them in an adenine-lacking medium.

Results: The first comprehensive mutational landscape of the Sup35N QN-rich region shows that 47% of possible single amino acid substitutions significantly reduce the nucleation of new aggregates. This reduction is more drastic in the central part of the mutagenized region (residues 17-25), suggesting a primary role of these residues in nucleating endogenous Sup35**.** What is more, our data matches previous work where the Sup35 17-25 segment was proposed to regulate species-specific *in vitro* and *in vivo* aggregation.
This dataset also reveals that ~4% of the possible single amino acid changes significantly boost Sup35 nucleation, especially substitutions to aromatic residues outside the 17-25 region.

Conclusions: By systematically quantifying the effect of 740 mutations in the QN-rich domain of Sup35 we determined the compatibility of each mutation with an effective nucleation of the wild-type Sup35. Thanks to this dataset, we identified a nine-residue segment crucial for this process, as well as mutants that increase Sup35 nucleation, gaining mechanistic insights on the nucleation of this model system and how prion species barriers can be overcome. Our work also evidences that comprehensive mutant libraries are an exceptional tool to explore prion sequences that help us address the multiple challenges encountered when studying prion-related questions.

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Grant number: PRE2019-088300, LCF/PR/HR21/52410004

Theme:

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	X
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Assessment of olfactory swab procedure performed by non-otolaryngologists for improving and simplifying human prion disease diagnosis.

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Aims:

RT-QuIC assay in cerebrospinal fluid (CSF) or "other tissues" has consistently improved the diagnosis in patients with prion disorders. Although CSF remains the gold standard for detecting potentially treatable conditions, we showed that the diagnostic accuracy of RT-QuIC in olfactory mucosa and CSF is comparable and that it is increased nearly to 100% when in the same patient both CSF and OM are tested (Bongianni et al. 2017). However, the requirement of an otolaryngologist limited an extended utilization of the procedure. In this study, olfactory swabbing will be performed by neurologists or non-ENT personnel to explore the reliability of the procedure. Sensitivity and specificity will be determined by analyzing CSF and OM samples obtained from the same patient by RT-QuIC.

Materials and Methods:

Nasal swabbing (NS) has been performed by neurologists or non-ENT personnel using a nasal swab (FloQBrush, Copan, Brescia) in 17 patients with sporadic CJD. As supportive investigation, 14 of these patients underwent lumbar puncture, and all samples were analyzed by RT-QuIC.

Results:

RT-QuIC was positive in 15/17 OM samples from sCJD patients (88%). No medical or traumatic complications linked to the procedure were observed. CSF was RT-QuIC positive in all 14 patients (100%).

Conclusions:

Here we showed that NS is a non-invasive and simply procedure with a high diagnostic performance which should be performed in the absence of otolaryngologists. This is relevant for an accurate and prompt clinical diagnosis of prion disorder, for a solicitous communication of the diagnosis to the family obviating purposeless investigations and for the availability of a tissue suitable for clinical trial. Thus, the diagnostic accuracy of olfactory mucosa collected by non-otolaryngologists should be explored in patients with sporadic and genetic prion disorders to determine the reliability of this approach.
We are aware that these preliminary data need to be confirmed in larger cohort of patients to be validated.

Acknowledgement:

We would like to thank Santina Castriciano for donating the flocked nasal swabs (FLOQBrushTM, Copan Italia, Spa, Brescia, Italy). We deeply thank Associazione Luca Nuti Onlus and families of CJD patients for their generous donations.

Theme (X)
Neuropathology of prion diseases
Functional protein aggregation in yeast and mammalian systems
Protein structure, function, conversion, and dysfunction
Spreading of pathology in prion-like disorders
Pathogenic mechanisms in prion and prion-like diseases
Animal prion diseases
Biomarkers for prion and other neurodegenerative diseases (X)
Therapeutic approaches for prion and prion-like diseases

Application of tear fluid as a less-invasive body fluid to diagnose prion disease cases via RT-QuIC

Matthias Schmitz*, Susana Correia*, Peter Hermann and Inga Zerr
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***equal contribution**

Abstract

Aims: In the present study, we aimed to modify the experimental conditions of the RT-QuIC for the amplification and detection of prion protein scrapie (PrP^{Sc}) in less invasive body fluids of prion disease patients, such as tear fluid.

Materials and Methods:

For the detection of PrP^{Sc} in tear fluid of prion disease patients, we needed to increase the sensitivity of the assay. Therefore, several recombinant PrP exhibiting PRNP-DNA sequences form different species, were tested for their seeding conversion efficiency in the RT-QuIC. Finally, we selected a sensitive recombinant PrP with the human PRNP sequence containing the E200K mutation (rec PrP FL Hu E200K), as substrate for the RT-QuIC. Our study cohort consisted of patients with sporadic Creutzfeldt-Jakob disease, patients with genetic prion diseases, pre-clinical PRNP mutation carriers and control donors.

Results:

We succeeded in amplification and detection of PrP^{Sc} in tear fluid of prion disease patients.

The calculation of the accuracy of the tear fluid RT-QuIC resulted in a good diagnostic sensitivity and specificity, which is comparable to CSF RT-QuIC. When we analyzed tear fluid from pre-clinical familial PRNP mutation carriers, we were able to detect PrP^{Sc}, suggesting the applicability of the tear fluid RT-QuIC as an early, non-invasive and potentially pre-symptomatic test.

Conclusion: Our study demonstrated that the RT-QuIC assay can be applied to diagnose prion disease patients using less-invasive tear fluid in the diagnosis of prion diseases. The new test opens new avenues for the analysis of pre-clinical patient samples or follow-up studies to evaluate the efficiency of a therapeutic intervention in future.

Theme: Biomarkers for prion and other neurodegenerative diseases

A humanised cell line propagating *bona fide* human variant Creutzfeldt-Jakob disease prions.

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Aims:
The ultimate aim of this project is to develop a high-throughput and highly sensitive cell-based bioassay which can detect pre-clinical levels of infectious vCJD prions in human tissues and biofluids. This required the development of a CAD5 cell derivative susceptible to 10^7 dilutions of vCJD infected human homogenates and conducting a mouse bioassay to confirm these cells were indeed propagating *bona fide* vCJD prions.

Materials and Methods:
Murine CAD5 cells were silenced for their endogenous mouse prion protein using CRISPR /Cas9-mediated mutagenesis and reconstituted with human prion protein containing methionine at amino acid 129. Several subclones were screened with vCJD inocula for infectivity, to identify a clone that was reproducibly susceptible to infectivity and could be readily quantified using a human prion assay. Pooled lysates from CAD5-PrP$^{-/-}$HuPrP M129 cells exposed to two MM homozygous vCJD-infected inocula were inoculated into FVB mice. Mice were monitored for clinical signs of prion disease and at the experimental end-point brain tissue was harvested and underwent histological and western blot analysis to determine *bona fide* vCJD infectivity.

Results:
Nine subsequent rounds of single cell cloning has identified a CAD5 cell clone that is susceptible to 10^7 dilutions of vCJD infected brain homogenates. These cells are susceptible to MV heterozygous vCJD infected brain inocula as well as multiple MM homozygous vCJD inocula.
Histopathological and immunoblot analysis for PrP^{Sc} in brains of mice infected with vCJD innocula and vCJD prions propagated in the CAD5- derived cells were identical, indicating the same prion strain was present in the infected cells and the original human brain homogenate.

Conclusions:
We have successfully produced a humanised cell line that reproducibly propagates *bona fide* human prions. These cells provide a potential tool to investigate therapeutics, factors associated with human prion cell strain and tropism and infectivity. They will now be taken forward to develop a high-throughput and highly sensitive cell-based bioassay which can detect pre-clinical levels of infectious vCJD prions in human tissues and biofluids.

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Grant number: PR-R17- 0916- 23004

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	X
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

General and Biomarker Cerebrospinal Fluid Findings in Prion Disease and other Rapidly Progressive Dementias

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University of California, San Francisco (UCSF), Department of Neurology, Memory and Aging Center, San Francisco, CA, USA.

Aims: To analyze the spectrum of basic and biomarker CSF findings in our RPD cohort and compare findings between symptomatic prion disease and non-prion RPD (npRPD) cases.

Materials and Methods: Retrospective cohort study of patients with RPD referred to the University of California San Francisco (UCSF) Memory and Aging Center (MAC) and evaluated in person or through medical record review. Sporadic CJD (sCJD) cases were included if they either met definite sCJD criteria or met UCSF symptom sCJD criteria and had a brain MRI and/or EEG (periodic sharp wave complexes) indicative of sCJD (probable sCJD). Genetic prion cases had *PRNP* mutations and were symptomatic at the time of data collection. npRPD cases met strict inclusion criteria. Data were extracted from our MAC RPD database. Statistical analyses were performed in R-studio.

Results: The cohort included: sCJD (n = 591), npRPD (n = 271), familial CJD (fCJD, n = 42), Gerstmann-Sträussler-Scheinker syndrome (GSS, n = 22), and Fatal Familial Insomnia (FFI, n = 4) for whom we had documented CSF data in our RPD database. 22% of the sCJD and 28% of npRPD cohort had elevated CSF total protein. CSF glucose of the sCJD cohort was slightly higher than npRPD (median (IQR): 66.0 (12) vs. 63.0 (13), p=0.0016). Surprisingly, ~5% of the sCJD cohort had leukocytosis (>5 WBCs;17% of npRPD), ~5% had elevated IgG index (>0.7 mg/dl; 14.3% of npRPD) and ~5.0% had elevated OCBs (>1; 16.7% of npRPD). The sensitivity, specificity, and AUC of sCJD vs. npRPD for t-tau (sCJD n=224; npRPD n=101) was 78.1%, 87.1% and 89.5%; for NSE (sCJD, n=161; npRPD, n=85) was 66.5%, 90.6% and 86.1%; for 14-3-3 (sCJD, n=283; npRPD, n=114) was 64.0, 85.1%, and 74.6%; and for RT-QuIC (sCJD, n=98; npRPD, n=17) was 85.7%, 94.1%, and 89.9%. For a subcohort with RT-QuIC, 14-3-3, and t-tau available from the same CSF sample (sCJD, n=94; npRPD, n=15), t-tau had the highest AUC, 93.2.

Conclusions: sCJD cases can have abnormal inflammatory markers and elevated protein. t-tau has high diagnostic accuracy, although not as specific as RT-QuIC, but might be used in conjunction with MRI and in place of 14-3-3 to diagnose prion disease.

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Acknowledgement: Our patients and their families

Theme	(X)
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Functional protein aggregation in yeast and mammalian systems	
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Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	x
Therapeutic approaches for prion and prion-like diseases	

Tracking Longitudinal Change in Presymptomatic Genetic Prion Disease

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Aims: Identify potential biomarkers for future treatment trials in the presymptomatic phase of genetic prion disease (gPrD).

Materials and Methods: Our preliminary data suggested that such biological changes can be measured in Presymptomatic gPrDs. Without formal funding, we followed participants in an ad hoc manner (less than annually) for more than 15 years and evaluated ~110 participants. After obtaining NIH R56/R01 funding in September 2018, we have followed asymptomatic carriers and non-carrier controls approximately annually in a more systematic manner using a standardized assessment battery over a 2-day visit.

Results: Approximately 143 gPrD participants from 54 families had research visits from 2008-2023, including 50 presymptomatic gPrD participants being seen through our NIH grant, “Tracking longitudinal change in presymptomatic genetic prion disease,” period. These 50 participants are from 29 families representing 6 of the more common PRNP mutations. 70% of these 50 participants are mutation carriers, 30% noncarriers.

As of April 2023, 26/50 participants have had serial research visits, 18 of whom also had at least one visit prior to the NIH funding period. These 50 participants had a total of 92 visits conducted prior to and during the grant period with the following collection rates: 3T Brain MRI, 96%; neuropsychological testing, 97%; CSF, 80%; blood for DNA, 100%; plasma collection, 91%; serum collection, 72% (began in 2018); RNA collection 99%, genetic counseling, 75%; detailed history and neurological exam, 99%; complete battery of informant measures, 60%; quantitative motor testing (qMotor), 78%; OCTs, 86%; and skin biopsies, 21% (began 2022). Analysis is beginning and recruitment continues. Preliminary analysis suggests we are identifying potential early biomarkers in the presymptomatic phase of gPrD. Furthermore, some PRNP mutation carriers have been found to have positive CSF biomarkers (specific and non-specific) even years before likely or known clinical onset.

Conclusions: Long-term observational studies in presymptomatic gPrD are feasible. Serial evaluations are continuing, and we expect we will identify more potential biomarkers of change over the course of presymptomatic gPrD. Information collected in this study will be important for developing clinical trials in presymptomatic gPrD.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	x
Therapeutic approaches for prion and prion-like diseases	

Fatal familial insomnia in China

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Aims: To summarize the clinical and genetic features of patients with Fatal familial insomnia (FFI) in China.

Materials and Methods: We collected clinical and genetic data from 13 FFI patients admitted to Xuanwu Hospital in Beijing, and 49 Chinese FFI patients / 85 from Western countries reported in the literature who with detailed information. Clinical and genetic features of Chinese FFI patients were summarized and compared with that of Western countries.

Results: Among the 13 patients at Xuanwu Hospital, 61.5% (8/13) had a positive family history, with an average age of 49.23 ± 13.10 years. The male-to-female ratio was 0.86:1. Eleven patients died with a mean survival time of 11.8 ± 3.08 months. All patients (13/13) exhibited sleep disturbances, rapidly progressive dementia, and autonomic symptoms. No high signal and PSWCs was found on Diffusion-weighted imaging (DWI) and electroencephalography (EEG) (13/13). Skin biopsy and CSF RT-QuIC were done in 2 patients, and all were positive. CSF neurofilament light (NFL) levels were measured in 2 patients, and all were elevated. Cortisol/melatonin circadian rhythm disappeared in 100% (4/4) of the patients.

Among all Chinese patients, a shorter disease duration (11.46 ± 5.97 vs. 14.43 ± 9.78 months) and a higher rate of positive CSF 1433 protein (56.7% vs. 18.2%) were found than Westerners. The genotype of 129 MM and MV was 1/61 and 61/21 in Chinese and Westerners. Currently, there are no specific interventions for FFI. Agomelatine and a ventilator might help.

Conclusion: FFI in China has a shorter disease duration, a higher positivity rate of 14-33 protein in cerebrospinal fluid, and a significantly lower proportion of the MV genotype at codon 129 than Westerners. Given the rarity of FFI, future studies should continue to expand the sample size to clarify the unique characteristics of the Chinese FFI population.

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Theme: Biomarkers for prion and other neurodegenerative diseases

Title: Probing Neuronal Hypersensitivity via Cross-Linking of Cellular Prion Protein and Anti-PrP Antibodies

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Aims: The neurotoxic effects of anti-PrP antibodies through cross-linking with cellular prion protein (PrPC) have been reported, but the underlying molecular mechanisms and the role of activated proteins in the apoptotic pathways leading to neuronal death remain poorly understood. Previous studies implicated proteins such as apolipoprotein E, cytoplasmic phospholipase A2, prostaglandin, and calpain in anti-PrP antibody-mediated apoptosis; however, these proteins also play a role in allergy. This study aimed to investigate whether cross-linking PrPC with anti-PrP antibodies elicits a neuronal allergenic response.

Materials and Methods: We initially predicted the allergenicity of epitope sequences associated with neurotoxic anti-PrP antibodies using allergenicity prediction servers. Subsequently, we examined whether treatment of mouse primary neurons (MPN), neuroblastoma cells (N2a), and microglia (N11) cell lines with anti-PrP antibodies induced a neuronal allergenic response.

Results: In-silico studies revealed the allergenic nature of both tail and globular epitopes. Binding regions containing epitopes of previously reported neurotoxic antibodies, such as ICSM18 (146-159), ICSM35 (91-110), POM 1 (138-147), and POM 3 (95-100), led to the activation of allergenic-related proteins. Treatment of N2a cells with anti-PrPC antibodies resulted in the identification of four neuronal allergenic-related proteins compared to untreated cells. Furthermore, treatment of N11 cells with anti-PrPC antibodies followed by co-culture with N2a cells identified eight allergenic-related proteins. Similarly, antibody treatment of MPN cells and co-culture with antibody-treated N11 cells revealed ten and seven allergenic-related proteins, respectively, compared to untreated cells. A comparison with 3F4 antibody treatment showed five and four allergenic-related proteins, respectively. Notably, the allergenic effects were more pronounced when antibody-treated microglia were co-cultured with the neuroblastoma cell line. Co-culture of N2a or MPN cells with N11 cells treated with anti-PrP antibodies resulted in significant accumulation of nitric oxide (NO) and interleukin-6 (IL-6) but not tumor necrosis factor-alpha (TNF-α) in the cell culture media supernatant.

Conclusion: This study demonstrates, for the first time, that binding of anti-PrP antibodies to PrPC triggers a neuronal hypersensitivity response. Furthermore, it highlights the crucial role of microglia in mediating an IgG-mediated neuronal hypersensitivity response. These findings underscore the importance of considering allergenic assessment of therapeutic antibodies for neurodegenerative disorders to ensure the development of safe and targeted biotherapeutics.

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Murine astrocytes display differential susceptibility to prion strains.

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Aims: Assess the roles of regional identity and differential susceptibility to prion strains using primary astrocyte cultures exposed to prions *in vitro*.

Materials and Methods:

i) Primary astrocyte isolation from cortex and cerebellum of WT and *Prnp* KO C57BL/6J p0-p4 pups
ii) *In vitro* infection with 22L, RML, or Me7 prion strains
iii) Western blotting for PrP (with and without PK digestion), GFAP
iv) Immunofluorescence (IF) for PrP, GFAP

Results: Glia were isolated from the cortex and cerebellum of WT and *Prnp* KO C57BL/6J pups. Analysis by western blot and IF revealed that approximately 80% of the isolated glia were astrocytes. Glia were exposed to 0.5% infected brain homogenate from 22L, RML, and Me7 infected brains for 24 hours and allowed to grow for 14 or 21 days before lysing and assessing infection through western blotting. After 14 days, both cortical and cerebellar glia were found to be infectible by 22L. However, even after 21 days, no PK-resistant material was detected for RML or Me7. These results indicate that cultured astrocytes propagate prions in a strain specific manner. There was no significant difference in PK resistant material between cerebellar and cortical glia. Additional experiments are underway to explore the prion susceptibility of astrocytes from other regions and subregions of the brain.

Conclusions: We have successfully created an *in vitro* model for astrocyte prion infection. This model will allow us to characterize the cell biology of prion propagation in astrocytes and will help us understand the regional susceptibility of the brain to different prion strains. Because astrocytes are now understood to possess strong regional heterogeneity, and vastly outnumber neurons in the brain, their role in strain susceptibility may be more significant than we currently appreciate. Our experiments will also permit us to explore neuron/astrocyte interactions, which are crucial to the pathology of prion diseases.

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Acknowledgement:

Theme	**(X)**
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	X
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Prion 2023

The role of unstructured domains of yeast Sup35 prion protein in liquid-liquid phase separation

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Aims: Formation of membranelles biomolecular condensates through liquid-liquid phase separation (LLPS) or gelation is one of approaches to intracellular compartmentalization. LLPS frequently results from intermolecular interactions of proteins, containing intrinsically disordered regions (IDR). Sup35 prion protein, a translation termination factor (eRF3) of yeast *Saccharomyces cerevisiae* has been shown to form both transmissible amyloid fibrils and reversible biomolecular condensates. The Sup35N region, containing IDRs, is sufficient for both LLPS and amyloid aggregation, however the roles of specific regions of N-domain in LLPS remained uncertain. The goal of this study is to determine sequence elements responsible for phase separation.

Materials and Methods: Sup35N, Sup35NM (containing the anti-aggregation middle, M domain) and Sup35NM derivatives with various deletions within the N-domain were fused to the yellow fluorescent protein (YFP) and then overproduced in the *S. cerevisiae* cells, lacking pre-existing prions. This was followed by the analysis of the ability of these derivatives to produce globular liquid condensates.

Results: Contrary to some previous reports, LLPS of overproduced Sup35N-containing constructs can be detected at near neutral pH. Various deletions within Sup35N counteract phase separation, with the deletion of the region of oligopeptide repeats, located between amino acid positions 40 and 97, exhibiting the strongest impact.

Conclusions: The region of oligopeptide repeats of Sup35N is playing a crucial role in liquid-liquid phase separation.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	X
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	

Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Uncovering the assembly mechanism involved in the formation of transient oligomeric species at the initial stages of prion protein pathogenic conversion

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Aims: Intermediate conformations forming during the conversion of the α-helix-rich cellular prion protein (PrP^C) form into the β-sheet–rich or fibrillary form (PrP^{Sc}) are key drivers in the misfolding process. Our prior research demonstrated that the truncated HuPrP(90-231) misfolding pathway occurs through the formation of a β-enriched intermediate state (β-PrPI) involved in the initial stages of PrP^C fibrillation. However, the mechanism by which β-PrPI recruits the monomers to form higher order amyloid structure is currently unknown. In this study, we aim to provide, at atomic resolution, the accurate structural characterization of the oligomeric species driving the fibril aggregation process activated by the stable β-enriched intermediate state (β-PrPI).
Materials and Methods: ^{15}N–^{13}C labeled truncated HuPrP(90–231) was investigated by solution Nuclear Magnetic Resonance (NMR). All NMR experiments were carried out at low temperature (15 °C) in order to quantitatively probe exchange dynamics between interconverting states by using a combination of Chemical Exchange Saturation Transfer (CEST) experiments in the absence and presence of β-PrPI-oligomers.
Results: By analyzing CEST saturation profiles of the residues surrounding the native β-sheets and the first a-helix, the kinetic and thermodynamic proprieties of human prion conformational equilibria were obtained. In according with previous results collected at 25 °C, CEST data indicate that in absence of oligomeric species, at low temperature, the native monomeric state rapidly interconverts with a minor conformational. On the contrary, in the presence of transient oligomeric β-PrPI- species, CEST analysis demonstrate that a great number of residues undergo structural changes during the assembly mechanism
Conclusions: High-resolution description of the structural rearrangements involved in the formation of transient oligomeric species that in turn activate the amyloid assembly mechanism will allow to understand the molecular machinery governing the prion misfolding associated with neurodegenerative diseases.

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	(X)
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Prion 2023 abstract title:
Assessing the Zoonotic Potential of North American and Scandinavian CWD Prions Through the Use of Non-Cervid Recombinant Prion Proteins in Real-time Quaking Induced Conversion

Authors:
Nathaniel D. Denkers[1], Sylvie L. Benestad[2], Glenn C. Telling[1], Edward A. Hoover[1], Candace K. Mathiason[1]

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[2]Norwegian Veterinary Institute, As, Norway

Aims: Chronic wasting disease (CWD), the invariably fatal prion disease of cervid species, continues to spread across North American (NA) and Scandinavia (Nordic). Preliminary studies suggest the emergence of these outbreaks are independent. As the characteristics and pathogenesis of these CWD prion isolates appears different, there is renewed concern regarding zoonotic transmission. The purpose of these studies is to assess the ability of NA and Nordic CWD prions to convert non-cervid species recombinant prion proteins using real-time quaking induced conversion (RT-QuIC) to better understand their potential transspecies and zoonotic capabilities.

Materials and Methods: Truncated recombinant bank vole (rtBVPrP) and hamster (rtHaPrP) prion proteins (90-231), expressed in *E. coli* BL21 cells, were generated for use as substrate in RT-QuIC. Serial dilutions (10^{-3}–10^{-8}) of CWD-positive and negative brain homogenates from NA white-tailed deer (wtd) (n=2) and Nordic reindeer (n=3) were assayed using each protein. Specificity and sensitivity were established based on reaction rates and end-point titration.

Results: Both recombinant proteins were excellent substrates, efficiently generating CWD specific amyloid seeds via RT-QuIC in NA and Nordic CWD isolates. Isolate titration data revealed the presence of higher prion concentrations in NA wtd isolates, with detectable seeding activity demonstrated from 10^{-3}-10^{-7}, than in Nordic reindeer isolates with detection from 10^{-3}-10^{-5}. Furthermore, amyloid reaction rates were higher in NA wtd when compared to Nordic reindeer (10^{-4}-10^{-7}).

Conclusions: While our initial results indicate a possible difference in North American and Nordic CWD isolates, experimental design modifications are required to gain more accurate outcomes. Primarily, matching brain regions (hind vs mid) for each isolate, as well as expanding non-cervid recombinant protein substrates to include bovine, ovine, and human recombinant proteins. Determining the transspecies and zoonotic potential of Nordic CWD will shape our understanding of the non-cervid reservoir and public health risks associated with this relatively recent discovery.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	(X)
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Chasing neuronal-exosomes for early diagnosis of Fatal Familial Insomnia

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Aims: Exosomes are small extracellular vesicles (EVs). Due to their property to cross the blood-brain barrier in a bidirectional manner, they can serve as minimally invasive liquid biopsies with extremely high diagnostic and therapeutic potential. Early diagnosis of fatal familial insomnia cases in easily accessible peripheral bio fluids is crucial to monitor disease progression and to assess potential therapies. Accordingly, we aimed to find out EV-based proteomic markers that affect the normal physiological functions prior to neurodegeneration.

Materials and Methods: We isolated brain-derived extracellular vesicles (neuronal exosomes) from blood of FFI patients and age-matched healthy controls using a simple and ultrasensitive magnetic nanowire-based technique to carry out differential proteomics. The identified proteomic signatures will be tested as novel diagnostic targets in human plasma-derived neuronal EVs in healthy carriers and age-matched controls to establish risk factors and presymptomatic biomarkers.

Results: We successfully isolated circular vesicles with size range and morphological characteristics consistent with exosomes. Efficient enrichment of EV-fractions was validated according to the criteria of International Society for Extracellular Vesicles. Identification of two neuronal markers (L1CAM and NCAM) in EV-fractions confirmed the enrichment of neuronal EVs. In total, 337 protein groups were identified and quantified at a critical FDR of 1 % using DIA-mass spectrometry analysis. Among these proteins, 37 proteins were contained in the Top 100 exosomes proteins annotated in the ExoCarta database. There were 71 proteins that were significantly modified (Welch's t-test, FDR <0.05).

Conclusions: Here, we report an efficient and sensitive method for the isolation of neuronal exosomes from blood. The investigation of plasma-derived neuronal EV-proteomes provides biomarker candidates for investigation in minimally invasive peripheral fluids. The longitudinal read out of blood-derived brain EVs can be used to obtain analytical information about disease progression.

Funded by: Joint Programming Neurodegenerative Disease_2021

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	**x**
Therapeutic approaches for prion and prion-like diseases	

Optimization of a nanotechnology-based methodology to isolate neuronal extracellular vesicles from biological fluids

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#Equal contribution

Aims: Exosomes are small extracellular vesicles (EVs), with a size range of ~40–160 nm. In neurodegenerative diseases, neuronal-derived exosomes have been collected from blood, CSF, saliva and urine and several potential biomarkers are explored. However, the validation and standardization of EV-originated biomarkers remain a challenge. Our aim was to optimize a sensitive and reliable method to isolate neuronal exosomes from blood plasma for diagnostic purposes.

Materials and Methods: We optimized a magnetic nanowire-based protocol to isolate neuronal-derived exosomes from plasma samples. For this, antibody-cocktail conjugated magnetic nanowires were used, coated with two neuronal exosomal markers (L1-CAM & NCAM) and a surface exosomal marker (CD81). We validated the protocol using NTA, TEM and immunoblotting approaches according to the standards of International Society for Extracellular Vesicles.

Results: Neuronal-derived exosomes were successfully isolated from plasma samples using a nanowire-based protocol. We characterized the EVs in triplicates using negative staining electron transmission microscopy. We found extracellular vesicles, with a typical morphology of small EVs (round cup-shaped particles with a size < 250 nm). Additionally, our EVs were evaluated by NTA in triplicates. In the NTA analysis, the largest peaks were observed in the size range of 75 to 160 nm (average = 116 nm). The immunoblotting results allowed us to detect seven different exosomal markers (CD9, CD63, CD81, Flotiln-1, Alix, HSP70 & Annexin V). To confirm the enrichment of neuronal-origin of isolates, two putative neuronal markers (L1CAM and NCAM) were also confirmed.

Conclusions: To conclude, this new antibody-cocktail conjugated nanowire-based protocol targeting neuronal exosomes is a rapid, effective, and reproducible method for direct isolation of brain-derived extracellular vesicles from biological fluids. Multiple antibody combinations can be used to pull-down small amounts of circulating brain-derived EVs.

Funded by: Joint Programming Neurodegenerative Disease_2021.

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	**x**
Therapeutic approaches for prion and prion-like diseases	

Different chronic stress paradigms converge on endogenous TDP43 cleavage and aggregation

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Institute of Translational Pharmacology (IFT), Italian National Council of Reseaches (CNR, Consiglio Nazionale delle Ricerche), Rome, Italy.

Aims: The aim of this work was to analyze endogenous TDP43 mobilization, cleavage and aggregation state upon acute and chronic insults in order to evaluate which stress paradigms better recapitulate the course of the disease.

Materials and Methods: We applied a panel of acute and chronic stressors to human neuroblastoma SH-Y5Y cells. We opted to perform our experiments on a neuronal cell line without any genetic manipulation to keep the system as close a possible to the cellular physiological state. Effect of stress was evaluated by cell viability assaqys and solubility assays, immunofluorescence staining and flow cytometry was performed to detect Thioflavin positive structures.

Results: Chronic stress resulted in the formation of TDP43 cleavage products normally associated with pathology, whereas acute stress failed to induce fragment formation. Immunofluorescence and flow cytometry allowed the detection of cytoplasmic TDP43 associated with Thioflavin positive structures upon long-term stress.

Conclusions: Our results point toward a physiological role of TDP43 cleaved fragments, along with an increade in amyloid strctures only upon chronic treatment, suggesting a putative physiological stress response based on TDP43 aggregation.

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Increase in plasma pTau181 levels and decrease of executive functions in healthy carriers of E200K mutation

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Introduction:
This study investigated the relationship between pTau181 levels in plasma, expected symptom onset, and cognitive function in healthy individuals carrying the E200K mutation.

Methods:
Data from 48 individuals were analyzed, and the number of years to expected symptom onset (at the time of data and samples collection) was calculated based on a published model. Two groups were compared: the first group included individuals more than 2 years before expected onset (n=39) and the second group included individuals less than 2 years from expected onset (n=9).

Results:
Mean age was 54.8 years (SD=5.6) vs. 67.8 years (SD=3) ($p<0.001$), mean years of education was 14.4 (SD=1.8) vs. 12.7 (SD=3.2) ($p=0.03$), 54% vs. 78% females ($p=0.27$) for the first and second groups, respectively.

ANCOVA analysis was performed to compare pTau181 levels in plasma between the two groups, with age included as a covariate. The overall model was significant, indicating that the combination of the grouping variable and age explained a significant proportion of the variance in pTau181 levels (Adjusted R-squared = 0.310). The main effect of the grouping variable was also significant, with the second group having higher pTau181 levels than the first group (1.726 pg/ml vs. 1.026 pg/ml).

Furthermore, ANCOVA was conducted to compare cognitive function between the two groups, controlling for age and years of education. No significant differences were found in MoCA scores, TMT-A, and digit span. However, TMT-B and phonemic verbal fluency showed significant differences between the groups (TMT-B z-scores were -1.55 (SD=1.8) vs. -6.7 (SD=7.3), and phonemic verbal fluency z-scores were 0.09 (SD=1.24) vs. -1.37 (SD=0.89) in the first and second groups, respectively.

Conclusion:
healthy carriers of the E200K mutation may exhibit increased pTau181 levels in plasma and subtle changes in executive functions prior to symptoms onset.

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Granagard® consumption in a cohort of E200K mutation carriers: a single site observational study

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Genetic CJD is relatively common in Israel due to a cluster of E200K mutation carriers. Even though carriers are at high risk of developing the fatal disease, knowledge about precipitating factors and preventative disease-modifying agents is lacking. Granagard®, a nanoformulation of pomegranate seed oil was shown to delay disease onset and aggravation in TgMHu2ME199K mice modeling for E200K PrP CJD in humans. A cohort of genetic CJD patients (n=24, mean age = 59.22±8.1, 16 females) and healthy carriers (n=48, mean age = 56.73±7.27, 24 females) of the relevant mutation is followed up over the last 3 years at the CJD clinic in the Tel-Aviv Medical Center. Demographical, clinical, and biological data is collected and analyzed, aiming to identify candidate risk factors and disease modifiers, as well as to characterize a prodromal disease phase using biomarkers in blood and CSF.

In this observational sub-study, we aimed to investigate the prevalence and possible effect associated with Granagard® consumption. Ten out of 48 (20.8%) healthy carriers reported daily consumption of Granagard® over the previous 5 years, and none of the enrolled CJD patients reported Granagard® use. No side effects were observed or reported by the participants. Further surveillance for an extended period of time can facilitate the understanding of a potential beneficial effect of Granagard® in delaying conversion in E200K carriers.

Prion 2023 abstract title:
Searching for perspectives for a large collection of cryo-preserved CJD brain tissue

Authors:
Otto Windl 1, Norbert Buresch 2, Sigrun Roeber 3, Viktoria Ruf 4, Benjamin Englert 5, Thomas Arzberger 6, Armin Giese 7, and Jochen Herms 8

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Text of Abstract:
Over more than 20 years the Center for Neuropathology and Prion Research (ZNP) in Munich has been the German Reference Center für spongiform encephalopathies. In this function, the ZNP has collected cryo-preserved brain tissue from almost 1200 cases with prion diseases. More than 10% of those cases had inherited forms of the disease and carried a mutation in the coding region of the prion protein gene. This material was assembled with immense effort and cost and is stored at the ZNP. However, its maintenance requires considerable resource (e.g. the annual energy consumption for the minus 80 freezers is approx. 25000 kWh) and constant attention. As the demand for this tissue is very low, this is an unsustainable situation in the long term.
We would like to bring this situation to the attention of the community of prion researchers and suggest a way forward with the following main points:
(i) To gather knowledge about the main scientific questions that may justify keeping this material in times of limited resources.
(ii) To gather knowledge about other collections of cryo-preserved tissue of prion diseases in Europe in order to decide which parts of the collection in Munich might be particularly worth keeping.
(iii) To draw up a plan for the selection of small subset of well-characterized samples, which is representative of prion diseases in Germany, will support possible future research projects and should be kept for the next 20 years.
(iv) To inform the major stakeholders of the collection such as the legal owner, the past funders and the scientific community.
(v) To discard the cryo-material of the majority of cases in a safe and dignified manner in order to keep the demand on resources for maintenance at a minimum.

Theme	
Neuropathology of prion diseases	X
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Reducing the sulfation of neuron-derived heparan sulfate accelerates prion protein clearance and prolongs survival time in prion-infected mice

Patricia Aguilar-Calvo[1#], Adela Malik[1@], Daniel R. Sandoval[2&], Christopher Barback[3$], Christina D. Orrù[4], Heidi G. Standke[5], Olivia Thomas[5], Chrissa A, Dwyer[2¥], Donald P. Pizzo[1], Jaidev Bapat[1ε], Katrin Soldau[1], Ryotaro Ogawa[3], Mckenzie B. Riley[6], K. Peter R. Nilsson[7], Allison Kraus[5], Byron Caughey[4], Jeffrey J. Iliff[8], David Vera[3], Jeffrey D. Esko[2], and Christina J. Sigurdson[1,9,10*]

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Aims: Amyloid plaques in prion patients are enriched in heparan sulfate (HS), a main component of the brain extracellular matrix and vascular basement membrane. HS accelerates prion disease progression and enhances plaque formation through unclear mechanisms. Here we aim to define how HS sulfation modulates the spread, scaffolding and clearance of prion aggregates *in vivo*.

Materials and Methods: First, we analyzed the disaccharide composition of HS, including the level and pattern of sulfation, in prion-infected brains using liquid chromatography/ mass spectrometry. Next, we used *N-deacetylase, N-sulfotransferase1* conditional knock-out mice to reduce the sulfation of either neuronal (*Ndst1*$^{f/f}$*SynCre*$^{+}$ mice) or astrocytic (*Ndst1*$^{f/f}$*GFAPCre*$^{+}$ mice) HS and challenged mice with diverse subfibrillar and fibrillar prion strains. Finally, we measured the transport of radiolabeled monomeric recombinant PrP^{C} through the central nervous system using live positron emission tomography (PET).

Results: We found that HS bound to brain-derived mouse and human prions is highly sulfated, particularly at position N. Reducing HS sulfation through *neuronal Ndst1* deletion resulted in 40% longer survival and decreased parenchymal plaque formation in mice infected with fibrillar prions (mCWD). Prion fibrils were shorter and more soluble in *Ndst1*$^{f/f}$*SynCre*$^{+}$ brains, but prion conformation was not otherwise detectably altered. Additionally, fibrils were deposited in meningeal vessels and periventricular areas, suggestive of enhanced transport toward perivascular drainage pathways in *Ndst1*$^{f/f}$*SynCre*$^{+}$ mice. Live PET imaging revealed faster transit of PrP^{C} within the brain and spinal cord in *Ndst1*$^{f/f}$*SynCre*$^{+}$ mice. Notably, reducing the sulfation of astrocytic HS sulfation had modest to no impact on the prion disease caused by subfibrillar prions, depending on the strain.

Conclusions: Our results show how selectively downregulating the *neuronal* expression of a single HS biosynthetic enzyme accelerates prion spread through the extracellular space and slows fibril elongation, identifying a target for aggregate clearance.

Funded by: This study was supported by the National Institutes of Health grants NS069566 (CJS), NS076896 (CJS), AG061251 (PAC), and the CJD Foundation (CJS).

Acknowledgement: We thank Biswa Choudhury and Mousumi Paulchakrabarti at the UC San Diego GlycoAnalytics Core for outstanding technical support and mass spectrometry analysis, and the animal care staff at UC San Diego for excellent animal care. We thank Daniel Ojeda-Juárez for critical review of the manuscript.

Theme	(X)
Neuropathology of prion diseases	x
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

THEME: **Animal prion diseases**

Prion 2023 abstract title: Detection of infectious prions in tissues of feral hogs

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Chronic wasting disease (CWD) is the prion disease with the greatest potential for spreading, affecting at least seven cervid species. An essential feature of prions lies in their ability to infect some species and not others. This phenomenon, known as a species barrier, seems to be largely dictated by the similarities between the donor (infectious) and recipient prion protein (PrP) sequences. Considering this, some potentially susceptible carnivores are proposed to act as vectors of CWD transmission as they may get infected. Alternatively, predators or scavengers may not get infected but spread infectious particles after they cross their digestive tracts.

Aims: This project aims to identify the presence of infectious prions in feral hogs living in CWD-endemic areas.

Materials and Methods: To do this, PMCA seeding activity was analyzed on feral hogs tissues such as the brain and retropharyngeal and submandibular lymph nodes using homologous pig PrP substrate or heterologous deer PrP substrate. We further injected selected feral hogs tissues into mice expressing the cervid and porcine versions of the prion protein to assess their potential to transmit disease.

Conclusions: Our results show positive in vitro PrPSc detection using porcine and cervid substrates. The considerably higher detection using cervid substrate suggests that although feral hogs are exposed to CWD prions, disease transmission is inefficient. Bioassays confirmed these results and demonstrated that the infectivity carried by feral hogs is not enough to induce disease. In summary, these results suggest that feral hogs may play a role in disseminating CWD prions across the landscape.

Prion 2023

Title: Real-Time Herd-level Chronic Wasting Disease Surveillance using Environmental Prion Protein (ePrP) Sentinel Technology

Authors: Marc D. Schwabenlander[1,2], Gage Rowden[1], Corina Valencia Tibbitts[1], Catalina Picasso-Risso[1], Sarah C. Gresch[1], Nathan Conner[2], Erik Hildebrand[3], Patrick Hagen[3], Mitch Lockwood[4], Joseph Hediger[5], Michael J. Cherry[5], David G. Hewitt[5], Qi Yuan[6], Jason C. Bartz[6], Tiffany M. Wolf[7], **Peter A. Larsen[1,2]**

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Aims: In light of the continued spread of chronic wasting disease (CWD) in cervids across North America and Europe, there is an urgent need for the development of highly sensitive prion (PrP^{Sc}) detection technologies that will help combat CWD in new ways. Real-time quaking-induced conversion (RT-QuIC) has emerged as a powerful assay for routine PrP^{Sc} detection. Similar to environmental DNA (eDNA) advances for pathogen detection and species discovery in aquatic and terrestrial environments, our work investigates a rapid method for extracting and detecting environmentally deposited prions (ePrP) using RT-QuIC. Here, we show how ePrP sentinels placed within areas where cervids congregate (both wild and captive) can be leveraged for real-time CWD surveillance.

Materials and Methods: We deployed ePrP sentinels in both captive and wild white-tailed deer and elk herds, including known CWD positive herds. Sentinels were recovered after defined periods of time, swabbed, and tested via RT-QuIC using ePrP-adapted protocols.

Results: We detected PrP^{Sc} in natural settings using ePrP-specific swabbing and extraction methods in conjunction with RT-QuIC. The sentinel technology and ePrP detection methods were useful in both white-tailed deer and elk herds, demonstrating multi-species functionality. Additionally, CWD prevalence of the animals interacting with the sentinels coincided with intensity of PrP^{Sc} detection – for example in a herd with 1 of

12 IHC positive animals, 1 of 16 swabs were RT-QuIC positive, and in a herd with 13 of 19 IHC positive animals, 19 of 34 swabs were RT-QuIC positive.

Conclusions: Our findings indicate that real-time detection of CWD in white-tailed deer and elk herds is possible using environmental sentinels and ePrP RT-QuIC protocols. We posit that non-invasive methods for real-time surveillance and pro-active discovery of CWD infected animals will lead to novel management solutions, ultimately helping to slow the spread of the disease.

Funded by: Funding was provided by the United States Department of Agriculture, Animal Plant Health Inspection Service, the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR), and the Agricultural, Research, Education, Extension and Technology Transfer (AGREETT) Program at the University of Minnesota.

Theme: Animal Prion Diseases

The role of the soluble N-terminal domain (N1-PrP) of the prion protein in αSyn Aggregation and Seeding

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Aims: The aim of this study is to investigate the interaction between the N1 fragment derived from α-cleavage of the prion protein and α-Synuclein (αSyn). The study explores the effects of N1-PrP on the aggregation and amyloid formation of αSyn, as well as its potential role in modulating αSyn seeding activity.

Materials and Methods: In this study, we employed various *in vitro* and *in cellulo* methods to investigate a role of N1-PrP on the aggregation of αSyn-A53T and the formation of seeding-competent conformers. Our methodology involved Super-Resolution Structured Illumination (SR-SIM) and Lattice Structured Illumination Microscopy (Lattice-SIM), fluorescence recovery after photobleaching (FRAP), supported lipid bilayers (SLBs), and Atomic Force Microscopy.

Results: First, we studied the effect of recombinant N1-PrP on the conformational transition of soluble αSyn-A53T into fibrils/amyloids *in vitro*. Employing ThT assays and Atomic Force Microscopy, we could show that N1-PrP prevented the formation of long fibrillar structure and thereby, amyloid formation.

We then studied the seeding activity αSyn-A53T assemblies formed in the presence of N1-PrP. To address this, the different *in vitro* generated αSyn-A53T preparations were added to the media of cell lines stably expressing soluble αSyn-GFP. Formation αSyn-GFP aggregates were analyzed by SR-SIM. Indeed, we could observe that the interaction of N1-PrP with pre-formed αSyn-A53T seeds modulated the latter's seeding activity.

As another approach, we investigated the interaction of αSyn-A53T PFFs with full length PrP on Supported Lipid Bilayers (SLB). The imaging of the SLB model revealed that αSyn-A53T PFFs promoted the clustering of membrane-bound PrP, suggesting their role in facilitating PrP aggregation.

Conclusions: In conclusion, our study highlights a dual role of N1-PrP in the formation and toxic activities of aggregated αSyn. As a soluble fragment, N1-PrP has a protective activity in preventing the activity of seeding-competent conformers of pathological αSyn, while in the context of full length, membrane-anchored PrP^C, it mediates the interaction with αSyn and toxic signaling by PrP.

Funded by: Deutsche Forschungsgemeinschaft
Grant number: Germany's Excellence Strategy – EXC 2033 – 390677874 – RESOLV and TA 167/6-3.

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	X
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

A probable diagnostic marker for CWD infection in humans

Xu Qi, Liuting Qing, Manuel Camacho, Ignazio Cali, Qingzhong Kong. Department of Pathology, Case Western Reserve University, Cleveland, USA

Aims: Multiple in vitro CWD-seeded human PrP conversion experiments and some animal model studies indicate that the species barrier for CWD to human transmission can be overcome, but whether CWD prion can infect humans in real life remains controversial. The very limited understanding on the likely features of CWD infection in humans and the lack of a reliable diagnostic marker for identification of acquired human CWD cases contribute to this uncertainty. We aim to stablish such a reliable diagnostic marker for CWD infections in humans should they occur.

Materials and Methods: A couple of PrP^{Sc}-positive spleens were identified from humanized transgenic mice inoculated with either CWD or sCJDMM1. Prions in these spleens were compared by bioassays in cervidized or humanized transgenic mice. A couple of PrP^{Sc}-positive spleens from UK sCJDMM1 patients were also examined similarly as controls with no exposure to CWD.

Results: We have detected two prion-positive spleens in humanized transgenic mice inoculated with some CWD isolates. Such experimentally generated splenic "humanized" CWD prions (termed $eHuCWD^{sp}$) appear indistinguishable from prions in the brain of sCJDMM1 patients on Western blot. We compared $eHuCWD^{sp}$ with prions in the spleen from humanized mice infected with sCJDMM1 (termed $sCJDMM1^{sp}$) by bioassays in cervidized or humanized transgenic mice. Significantly, we found that $eHuCWD^{sp}$ can efficiently infect not only the humanized mice but also cervidized transgenic mice, and cervidized mice infected by $eHuCWD^{sp}$ produced PrP^{Sc} and brain pathology that are practically identical to those of CWD-infected cervidized mice. In contrast, $sCJDMM1^{sp}$, similar to prions from sCJDMM1 patient brains, is poorly transmissible in the cervidized mice.

Conclusions: Our data demonstrate that high transmissibility with CWD features of splenic prions in cervidized transgenic mice is unique to acquired human CWD prions, and it may serve as a reliable marker to identify the first acquired human CWD cases.

Funded by: NIH
Grant number: R01NS052319, R01NS088604, R01NS109532
Acknowledgement: We want to thank the National Prion Disease Pathology Surveillance Center and Drs. Allen Jenny and Katherine O'Rourke for providing the sCJD samples and the CWD samples used in this study, respectively.

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	X
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Fortuitous generation of a zoonotic cervid prion strain

Manuel Camacho, Xu Qi, Liuting Qing, Sydney Smith, Jieji Hu, Wanyun Tao, Ignazio Cali, Qingzhong Kong. Department of Pathology, Case Western Reserve University, Cleveland, USA

Aims: Whether CWD prions can infect humans remains unclear despite the very substantial scale and long history of human exposure of CWD in many states or provinces of USA and Canada. Multiple in vitro conversion experiments and in vivo animal studies indicate that the CWD-to-human transmission barrier is not unbreakable. A major long-term public health concern on CWD zoonosis is the emergence of highly zoonotic CWD strains. We aim to address the question of whether highly zoonotic CWD strains are possible.

Materials and Methods: We inoculated several sCJD brain samples into cervidized transgenic mice (Tg12), which were intended as negative controls for bioassays of brain tissues from sCJD cases who had potentially been exposed to CWD. Some of the Tg12 mice became infected and their brain tissues were further examined by Western blot as well as serial passages in humanized or cervidized mice.

Results: Passage of sCJDMM1 in transgenic mice expressing elk PrP (Tg12) resulted in a "cervidized" CJD strain that we termed CJD^{ElkPrP}. We observed 100% transmission of the original CJD^{ElkPrP} in transgenic mice expressing human PrP. We passaged CJD^{ElkPrP} two more times in the Tg12 mice. We found that such second and third passage CJD^{ElkPrP} prions retained 100% transmission rate in the humanized mice, despite that the natural elk CWD isolates and CJD^{ElkPrP} share the same elk PrP sequence. In contrast, we and others found zero or poor transmission of natural elk CWD isolates in humanized mice.

Conclusions: Our data indicate that highly zoonotic cervid prion strains are not only possible but also can retain zoonotic potential after serial passages in cervids, suggesting a very significant and serious long-term risk of CWD zoonosis given that the broad and continuing spread of CWD prions will provide fertile grounds for the emergence of zoonotic CWD strains over time.

Funded by: NIH
Grant number: R01NS052319, R01NS088604, R01NS109532
Acknowledgement: We want to thank the National Prion Disease Pathology Surveillance Center and Drs. Allen Jenny and Katherine O'Rourke for providing the sCJD samples and the CWD samples used in this study, respectively.

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	**X**
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Prion 2023 Abstract Title: Exploring Novel Immunodiagnostic Tools for CWD Prion Detection

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Aims: Chronic Wasting Disease (CWD) is a transmissible, highly infectious prion disease of cervids. CWD continues to spread throughout North American cervid populations, and cases are increasing in Nordic moose, red deer, and reindeer. Traditional diagnostic options for the rapid and reliable detection of CWD prions (e.g., IHC and ELISA) have numerous limitations and disadvantages. There is an urgent need to develop new diagnostic tools to facilitate new surveillance and management options for CWD. Several exciting immunodiagnostic assays have recently emerged that can be leveraged to advance global CWD surveillance efforts.

Materials and Methods: Here, we present two areas of research that focus on developing next-generation CWD immunodiagnostic assays. First, we use proximity ligation technology to leverage an ultrasensitive RT-PCR Protein Assay for immunodetection and relative quantification of CWD prions. When combined with portable RT-PCR platforms, the proximity ligation CWD assay becomes a powerful and sensitive field-based diagnostic option for real-time CWD surveillance. Second, we produced shark nanobodies, or single-domain variable new antigen receptors (VNARs), targeting mammalian prion protein (PrP). Shark VNARs exhibit various unique properties that separate them from traditional mammalian antibodies (e.g., IgG), thus facilitating the development of downstream next-generation CWD diagnostic tools

Results: Proximity ligation experiments resulted in the identification of four antibody combinations with PrP binding locations sufficient for RT-PCR-based amplification. Proof-of-concept for portable RT-PCR diagnostics was established with magnetic induction cycling technology. Six shark VNARs were produced having binding capacity for mammalian PrP.

Conclusions: Our work collectively reveals eminent promise for a new class of immunodiagnostic technologies that will expand CWD research opportunities and ultimately enhance surveillance efforts.

Funded by: The Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR)
Grant number: N/A

Acknowledgement: We thank Suzanne Stone and Marc Schwabenlander for logistical assistance within MNPRO labs.

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	(X)
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

PRION 2023 abstract title: **"Strain-specific trajectories of sporadic Creutzfeldt-Jakob disease propagation in the brain revealed by MRI applying a Subtype and Stage Inference model"**

Riccardo Pascuzzo[1], Alexandra L. Young[2,3], Neil P. Oxtoby[2], Lavista Osborn[4], Keisi Kotobelli[4], Mark L. Cohen[4,5,6], Lawrence B. Schonberger[7], Pierluigi Gambetti[5], Daniel C. Alexander[2], Brian S. Appleby[4,5,6,8], and Alberto Bizzi[1]

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Aims: Recent Magnetic Resonance Imaging (MRI) studies demonstrated that diffusion-weighted image (DWI) alterations, markers of prion protein misfolding, start focally in distinct brain regions (disease "epicenters") and spread according to subtype-specific trajectories. This study aimed to (1) group sporadic Creutzfeldt-Jakob disease (sCJD) patients with similar trajectories of DWI alterations applying a data-driven method, and (2) verify whether such groups correspond to different sCJD subtypes/strains.

Materials and Methods: DWI alterations of 563 autopsy-confirmed sCJD patients were prospectively evaluated by one neuroradiologist, blind to diagnosis. We adopted Subtype and Stage Inference (SuStaIn) model, an unsupervised machine-learning technique that uncovers population subgroups with distinct trajectories of biomarker (i.e., DWI) alterations. SuStaIn determined the optimal number of groups/clusters based only on imaging data (subtype/strain not included in the model) and simultaneously reconstructed the trajectory of DWI alterations for each cluster. Next, the association between clusters and sCJD subtypes/strains was assessed.

Results: SuStaIn identified 5 clusters of patients: DWI alterations spread from the cortical ribbon to the basal nuclei in three clusters and from the basal nuclei to the cortex in two. The 5 clusters correlated well with sCJD strain.

The first cluster included most patients with MM1 subtype: DWI alterations originated in the parietal and frontal cortices, then spread to the striatum, temporal cortex, and eventually reached the thalami and cerebellum. The other two clusters with epicenter in the cortex showed DWI alterations involving all cortical lobes before reaching the striatum and included most patients with MM(MV)2C and VV1 subtypes.

Patients with VV2 and MV2K subtypes formed the remaining two clusters with epicenter in the basal nuclei (striatum and thalamus, respectively) and a very late involvement of the neocortex.

Conclusions: In vivo diagnosis of sCJD subtype is feasible with MRI alone. Knowledge of the trajectory of lesion propagation may be used as a biomarker to inform patient management and select patients for clinical trials.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	X
Therapeutic approaches for prion and prion-like diseases	

Investigation of prion resistance to Elacridar

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Aims:
The emergence of drug resistance has hampered the development of anti-prion compounds that are effective *in vivo*. We report here on experiments designed to understand the mechanism of action of Elacridar, and the discordance between its *in vitro* and *in vivo* effects.

Materials and Methods:
-Cell based PrP^{Sc} clearance & toxicity assays
-*in vivo* drug treatment
-Histopathological analysis
-RT-QuIC
-Conformational stability assay

Results:
Elacridar is a potent anti-prion compound (EC_{50} of 700 nM) that can permanently cure ScN2a cells of infection and protect hippocampal dendritic spines from prion induced collapse. However, despite brain concentrations 20x above the EC_{50}, Elacridar had no effect upon RML incubation time, PrP^{Sc} deposition pattern, or RT-QuIC seeding activity. We investigated the possibility that Elacridar treatment resulted in the emergence of drug-resistant prions *in vivo*. The cell tropism of RML prions was unaltered by passage through Elacridar-treated mice, as judged by infection of a panel of cell lines used to distinguish prion strains. Moreover, these mouse-passaged prions were Elacridar-sensitive following infection of the cell panel. Conformational stability assays revealed that Elacridar-treated prions were less stable than vehicle-treated at 90 dpi but this difference was less prominent in prions at end stage. Interestingly, the anti-prion effects of Elacridar were significantly diminished when tested in non-dividing cells *in vitro*, including ScN2a cells treated with chemical inhibitors of cell division, as well as in terminally differentiated C2C12 myotubes.

Conclusions:
These experiments suggest that prions adapt *in vivo* to overcome the anti-prion effects of Elacridar, similar to observations made of quinacrine resistant prions. Propagation in non-dividing cells *in vitro* may facilitate the emergence of Elacridar resistance, mimicking what may occur *in vivo*. Ongoing experiments are examining the anti-prion effects of Elacridar using prion-infected brain slices and cultures of primary astrocytes. Despite its lack of therapeutic effect *in vivo*, Elacridar may be a useful tool to understand basic mechanisms of prion propagation and toxicity.

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Acknowledgement: We thank David Westaway for providing moPrP expressing RK13 cells, Ina Vorberg for L929 cells, Charles Weissman for PK1 cells, and Joel Watts for CAD5 cells. We thank Byron Caughey and Brent Race for prion stocks and the bvPrP expression plasmid.

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	X

Deciphering the pathological significance of self-propagating Aβ strains in different animal models

Nazaret Gamez, Claudia Duran-Aniotz, Ines Moreno-Gonzalez, Ruben Gomez-Gutierrez, and Rodrigo Morales.

Background: Alzheimer's disease (AD) is an heterogenous disorder characterized by the accumulation of amyloid-beta (Aβ) and tau. One possible explanation for the clinical and pathological variation in AD lies in the presence of distinct conformational strains of Aβ. Numerous studies provide compelling evidence for the existence of such strains as well as their ability to template their conformations in a prion-like manner. However, the interaction of such Aβ strains with different hosts has yet to be thoroughly examined. Here, we examined the host-seed interaction of different human brain-derived and synthetic misfolded Aβ strains in two different mouse models of amyloid pathology. We specifically explored the potential differences in amyloid propagation and pathological manifestations considering Aβ strains and animal models as variables. **Methods**: 50-day-old Tg2576 mice were intra-cerebrally challenged with mouse or human brain-derived Aβ seeds, or synthetic Aβ strains. Age-matched APP/PS1 mice were intra-cerebrally challenged with the biological Aβ seeds as well. Cerebral pathology was assessed in 300-day-old Tg2576 mice, and in 180-day-old APP/PS1 mice. **Results**: Two structurally defined synthetic misfolded Aβ strains induced remarkably different pathological features in Tg2576, including different rates of aggregation, tropism to specific brain regions, and differential recruitment of Aβ40/Aβ42 peptides. Moreover, the administration of human brain homogenates derived from AD patients, displaying unique patterns of amyloidosis, seeded distinct phenotypes in challenged APP/PS1 mice, as evaluated by their seeding activity, vascular pathology, and reactivity to thioflavin-S. Finally, we observed that the same human brain homogenate induced a different cerebral Aβ pathology when inoculated into Tg2576 and APP/PS1 mice. **Conclusion**: Our findings support the concept of Aβ strains and demonstrate that they may drive different clinical and pathological outcomes in humans. Our work highlights the contribution of the host in pathological characteristics. Future research could lead to successful personalized diagnostic approaches and treatments.

Funded by NIH 1R01AI132695

Theme: Spreading of pathology in prion-like disorders

Intra-lingual exposure of Aβ aggregates as an effective mean to induce brain amyloidosis.

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Background: Misfolded amyloid-β (Aβ) peptides self-propagate and spread akin to infectious prions. Compelling evidence demonstrate that peripheral administration of Aβ seeds exacerbates brain amyloidosis in both susceptible experimental animals and humans. While the exact mechanisms governing the peripheral transport of Aβ seeds to the brain remain unclear, circulation and retrograde axonal transport have been proposed as contributing processes. Our previous work demonstrated that while the administration of Aβ seeds via eye drops was the most effective route in inducing Aβ pathology in Tg2576 mice, oral administration did not have any effect on brain pathology. Based on prion studies, prion inoculation into the tongue is considered as a more efficient pathway than oral ingestion, thus warranting further investigation. Here, we explored the role of two highly innervated tissues, the tongue and the nasal epithelium, in accelerating brain amyloidosis after the exogenous exposure to misfolded Aβ. **Methods**: 50-day-old Tg2576 animals were challenged by extra-nasal exposure or intra-lingual inoculation of old Tg2576-brain homogenate (BH) containing Aβ seeds. The estimated amount of administered $A\beta_{42}$ was 4.7 ng by each route. Animals were sacrificed at 300 days, brains were removed, and histological as well as biochemical analyses were performed. **Results**: The injection of Aβ seeds in the tongue, a highly innervated organ, significantly accelerated the accumulation of amyloid deposits in the brain of Tg2576 mice. Interestingly, the extra-nasal exposure of Aβ aggregates increased amyloid pathology in the olfactory bulb. **Conclusion**: Our results show that exposing highly innervated tissues to Aβ seeds accelerates Alzheimer´s disease-like neuropathological features, suggesting that Aβ peptides may be transported from the periphery to the brain by retrograde axonal transport. Research in this direction may be relevant on different fronts, including disease mechanisms, diagnosis, and risk-evaluation of potential iatrogenic transmission of Aβ misfolding.

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Theme: Spreading of pathology in prion-like disorders

Comprehensive analysis of cellular and pathological prion protein isoforms in different prion strains supports a neurotoxic role of membrane-anchored full-length PrP^{Sc}

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Aims: Prion strains cause a variety of clinical forms, involving different cells and brain areas, but sharing the neurodegenerative pathway that accompanies the replication and accumulation of prions in the CNS. The mechanisms causing neurotoxicity are still unknown. Loss-of-function could be caused by downregulation or changes in the proteolytic processing of PrP^{C}. Toxic gain-of-function mechanisms could be triggered by conformational variants of pathological PrP^{Sc}. The lack of studies investigating the complexity of physiological and pathological PrP isoforms in multiple prion strains hampered a deeper understanding of these phenomena.

Materials and Methods: We selected 4 human-derived and 3 animal-derived bank vole-adapted prion strains showing variable incubation periods, PrP^{Sc} conformations and neuropathology. We analysed PrP^{C} and PrP^{Sc} species in the brain of diseased voles by combining: i) solubility-based separation of PrP^{C} and PrP^{Sc} pools, ii) quantitative WB able to identify all PrP fragments by deglycosylation and epitope mapping, iii) discriminatory IHC. This allowed us to quantify PrP^{C} and PrP^{Sc} species in solution and to localize the main PrP^{Sc} fragments in situ. Finally, we correlated quantitative variations of PrP^{C} and PrP^{Sc} species with the incubation period of the different strains, as a proxy for neurotoxicity.

Results: PrP^{C} downregulation was observed in some, but not all strains, without obvious correlation with incubation periods. It mainly depended on a decrease of full-length PrP^{C} proteoforms (max 40%), rather than C1 and C2 proteolytic fragments. Total PrP^{Sc} levels did not correlate with neurotoxicity, while there was a correlation between long incubation periods and high relative levels of i) extracellular shed PrP^{Sc} and ii) intracellular N-terminal truncated PrP^{Sc} fragments.

Conclusions: Our findings suggest that shedding and N-terminal truncation of PrP^{Sc}, the latter possibly in the endosomal-lysosomal pathway, could be neuroprotective reactions to prion replication, supporting the hypothesis that membrane-anchored, full-length PrP^{Sc} plays a major role in neurotoxicity.

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Acknowledgement:

Theme:

Pathogenic mechanisms in prion and prion-like diseases

Evidence for preexisting substrains in a biologically cloned prion strain

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Aims: Prion strains are operationally defined as a heritable phenotype of disease under controlled transmission conditions. Treatment of rodents with anti-prion drugs results in the emergence of drug-resistant prion strains and suggest that prion strains are comprised of a dominant strain and substrains. While much experimental evidence is consistent with this hypothesis, direct observation of substrains has not been observed. The aim of this study was to determine if suppression of replication of a biologically cloned strain would allow for the emergence of substrains.

Materials and Methods: The biologically cloned drowsy (DY) strain of hamster-adapted transmissible mink encephalopathy (TME) was used in these studies. To investigate if DY replication is required for suppression of substrains we used protein misfolding cyclic amplification strain interference as described previously by this group. To investigate for prion substrains, DY PrP^{Sc} was selectively removed using extended proteinase K (PK) digestion or partial guanidinium denaturation and PK digestion. The resultant material was subjected to protein misfolding cyclic amplification and animal bioassay to probe for substrains.

Results: Here we show that replication of the dominant strain is required for suppression of a substrain. Based on this observation we reasoned that selective reduction of the dominant strain may allow for emergence of substrains. Using a combination of biochemical methods to selectively reduce drowsy (DY) PrP^{Sc} from biologically cloned DY TME infected brain resulted in the emergence of strains with different properties than DY TME.

Conclusions: The selection methods used here did not occur during prion formation, suggesting the substrains identified pre-existed in the DY TME-infected brain. DY TME is biologically stable suggesting that substrains exist under conditions where the dominant strain does not allow for substrain emergence. These observations suggesting that substrains are a common feature of prions.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	

Pathogenic mechanisms in prion and prion-like diseases	X
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Flexible N-Terminal Domain of the Prion Protein is Neurotoxic *in vivo*

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Aims: Prion diseases are fatal neurodegenerative disorders caused by conversion of physiological prion protein (PrP^{C}) to the pathological PrP^{Sc} conformer. Despite extensive research efforts, the detailed neurotoxic mechanism underlying prion diseases remains elusive. Previous studies have suggested that the N-terminus of PrP^{C} is neuroprotective. However, it remains unclear whether the N-terminus of PrP^{C} has a similar neuroprotective effect *in vivo*. Our study aims to study the biological effect of the N-terminus of PrP *in vivo*.

Materials and Methods: Because the N-terminus of PrP^{C} is naturally unstructured, it is difficult to express it at a sufficiently high level *in vivo* to determine its biological effect. We tested various approaches and successfully expressed it at a high level in mice.

Results: The expression of the N-terminus of PrP^{C} resulted in a very synchronous death in all mice, which is similar to prion disease. In addition, our study showed that the N-terminal PrP-caused neurotoxicity requires the presence of signal peptide, indicating that it exerts its toxic effect extracellularly. Moreover, we also found that the positively charged amino acid clusters and the octapeptide repeats in the N-terminus of PrP are essential for the toxicity.

Conclusions: The flexible N-terminus of PrP is highly neurotoxic rather than neuroprotective *in vivo*.

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	X
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Prion 2023 abstract title: Granagard administration prolongs the survival of endogenous and transplanted stem cells

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Hadassah University Medical Center, Jerusalem, Israel

Aims: We have shown previously that Granagard, a nanoformulation of pomegranate seed oil, can elongate the survival of neuronal stem cells transplanted into a genetic CJD model. Transplantation of autologous Mesenchymal stem cells (MSCs) to Multiple Sclerosis (MS) patients was shown to be beneficial but short lived due to the poor survival of the transplanted stem cells. We now aimed to show whether administration of Granagard to human MSCs transplanted EAE mice, representing a model of MS, can elongate the beneficial clinical effect of these cells.

Materials and Methods: Wt mice were immunized with MOG 33-35 at day 1 and boosted at day 35, resulting in two waves of EAE disease, before termination of the experiments at day 60. Mice were followed for disease scores daily and subsequently their brains were collected for analysis.

Results: Our results show that while in the initial EAE phase, hMSCs transplantation in both the presence or absence of Granagard mostly prevented disease, in the second phase, the transplanted hMSCs lost their clinical benefit unless Granagard was also administered. Accordingly, pathological studies at termination of the experiments identify hMSCs only in the brains of transplanted mice treated with Granagard. Furthermore, DCX and Nestin immunofluorescence, representing neurogenesis, were significantly increased by the combined treatment. We have shown previously that Granagard increased endogenous stem cell generation in both brains and muscles of CJD mice. Inhibition of EAE induced GFAP activation was observed in all Granagard treated mice but was significantly elevated by the combined treatment.

Conclusions: We conclude that Granagard, a safe food supplement, may prolong the clinical benefits of stem cell transplantation in diverse neurological diseases. The beneficial effect of Granagard is now tested in MS patients treated by hMSCs transplantation.

Funded by: Granalix Biotechnologies

Title: The anti-inflammatory capabilities of cellulose ethers by regulation of the NLRP3 inflammasome

Authors: Ryan Sayers[1,2,3,4], Tahir Ali[2,3,4], Sabine Gilch[2,3,4]

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Aims: Alzheimer's disease (AD) is a chronic, incurable neurodegenerative disease, and the major cause of dementia. AD is characterized by the buildup of misfolded protein aggregates, primarily amyloid beta (Aβ), in the brain. Neuroinflammation, a response of the innate immune system, is connected with AD pathogenesis as aberrant neuroinflammation mediates neurodegeneration and correlates with Aβ aggregation. The NLRP3 inflammasome protein complex is an integrator of this response, activating proinflammatory mediators that trigger neuroinflammation. Our group recently found that cellulose ethers (CEs) prevent Aβ aggregation, attenuate neuroinflammation, and rescue memory function in mouse models of AD. Our aim is to test if CEs have a direct anti-inflammatory effect and if this is linked to antagonization of the NLRP3 inflammasome response.

Materials and methods: An immortalized murine microglia cell line (BV2) was grown to 80% confluency and exposed to lipopolysaccharide (LPS) at 1 µg/mL for 4 hours to prime NLRP3 inflammasome, followed by treatment with ATP at 1 mM for 4 hours, for activation. This was conducted with or without CE exposure at 1% concentration for the duration. Cell lysates were collected and analysed for microglial activation using Iba-1 and NLRP3 inflammasome markers (Caspase-1, Asc1) by Western blots. Confocal microscopy was used to visualize localization of activated NLRP3, Iba1, and Caspase-1.

Results: Our preliminary results confirm elevated inflammatory and microglial activation markers in cells exposed to LPS and ATP. CE treatment decreased expression of these markers and of NLRP3 constituent proteins, indicating a rescue from an inflammatory response and antagonization of NLRP3 inflammasome activation.

Conclusion: Our results indicate that CEs have an anti-inflammatory effect *in vitro*. CEs are attractive for drug discovery as they are FDA-approved food/pharmaceutical additives, and thus offer a potential therapeutic to reduce neuroinflammation in protein-misfolding neurodegenerative diseases such as AD.

Funded by: Faculty of Veterinary Medicine, University of Calgary

Acknowledgement: Su Shim, Katsumi Doh-ura

Theme: Therapeutic approaches for prions and prion-like diseases

Norwegian moose CWD induces clinical disease and spleen-independent neuroinvasion in mice expressing cervid S138N prion protein

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Aims: Norwegian CWD prions were determined to be different strains than North American CWD. Moose and red deer strains are considered non-lymphotropic and represent cases of sporadic CWD. We aimed to analyse transmissibility and neuroinvasion ability to different *Prnp* genotypes. These include the S138N polymorphism found in reindeer/caribou, which has been associated with reduced susceptibility to North American CWD.
Material and Methods: Gene-targeted mice expressing cervid *Prnp* encoding either serine or asparagine at codon 138 were intracerebrally or intraperitoneally inoculated with brain homogenates from CWD-positive reindeer, moose, or red deer from Norway. Brains and spleens were analysed by proteinase-K (PK) digestion followed by immunoblot, real-time quaking-induced conversion assay and immunohistochemistry. PrP^{Sc} was further analysed using different antibodies to determine N-terminal cleavage sites.
Results: Previously, we have demonstrated that gene-targeted mice expressing 138NN or 138SN PrP did not develop clinical disease when inoculated with North American CWD isolates, but were subclinically infected. Here, we show that Norwegian moose CWD inoculated intracerebrally into gene-targeted mice homozygous for 138NN PrP breaks the transmission barrier and causes terminal clinical prion disease, with a survival time shorter than mice that express 138SS PrP. Moreover, analysis of PK-resistant PrP^{Sc} fragments (PrP^{res}) revealed two distinct patterns in clinical 138NN mice. Only 138SS-expressing mice were susceptible to clinical disease upon intraperitoneal infection. Despite absence of clinical signs, 138NN mice inoculated with Norway moose CWD harbored seeding activity in their brains. Surprisingly, seeding activity was not detected in their spleens. Red deer CWD was only transmissible to mice overexpressing 226E-PrP.
Conclusions: Gene-targeted mice expressing 138NN PrP are susceptible to clinical CWD, highlighting the critical role of strains in susceptibility to prion disease. PrP^{res} patterns suggest selection of distinct PrP^{Sc} conformers. Norway moose CWD can be neuroinvasive, using a route that is independent of spleen replication.

Funded by: Alberta Prion Research Institute, Margaret Gunn Endowment for Animal Research, Natural Sciences and Engineering Council of Canada (NSERC)

Theme	
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	x
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Fast Axonal Transport of PrP^{Sc}

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Aims: PrP^{Sc} spreads through defined neuroanatomical pathways, but the PrP^{Sc} transport mechanisms are unknown. Current studies indicate PrP^{Sc} slow axonal transport, but the methodology has low sensitivity and includes the confound of newly replicated PrP^{Sc}. Also, the role of PrP^{C} is debated, despite being necessary for PrP^{Sc} replication and disease propagation. In this study, we aim to:

1. Directly visualize and empirically measure PrP^{Sc} transport.
2. Measure PrP^{Sc} transport in $PrP^{+/+}$ and $PrP^{0/0}$ backgrounds to analyze the necessity of PrP^{C} in PrP^{Sc} transport.

Materials and Methods: RML scrapie, HY and DY TME, and 139H PrP^{Sc} strains were NaPTA purified and conjugated to Alexa Fluor 647 (AF^{647}). Purity and conjugation were confirmed by Sypro Ruby and fluorescent gels, respectively. Dextran-AF^{647} or PrP^{Sc}-AF^{647} was inoculated into $PrP^{+/+}$ or $PrP^{0/0}$ mouse sciatic nerves (ScN). ScN were immediately excised and placed into a FCS3 live cell chamber maintained at 37ºC with continual oxygenated glucose infusion and were imaged using a Leica SP8 multiphoton microscope (ex: 840 nm, em: 660-720 nm). Spectral scans and timeseries of particles were acquired in multiple ScN axons. Fiji/ImageJ plugin, Trackmate, was utilized to localize, track, and acquire particle velocities.

Results: RML, HY, DY, and 139H were successfully purified and conjugated to AF^{647}. Axons containing Dextran-AF^{647} or PrP^{Sc}-AF^{647} were successfully imaged in multiple nerve segments and individual nerves. Trackmate was able to detect particles along axons and measure velocities. A significant number of Dextran-AF^{647} and PrP^{Sc}-AF^{647} particles underwent fast axonal transport (2-5 µm/sec).

Conclusions: We were able to directly image purified, fluorescently tagged PrP^{Sc} in *ex vivo* ScN explant axons. All tested strains underwent fast axonal transport in $PrP^{+/+}$ and $PrP^{0/0}$ ScN, and had velocities comparable to Dextran, known to undergo fast axonal transport. This indicates PrP^{C} independent fast axonal transport of PrP^{Sc}, in direct contrast to previous studies.

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Theme	(X)
Neuropathology of prion diseases	

Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	X
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Toll-like receptor-mediated glial activation regulates prion replication in early stage of infection.

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Aims: Prion diseases are progressive neurodegenerative disorders affecting humans and various mammals. The prominent neuropathological change in prion-affected brains is neuroinflammation, histopathologically characterized by reactive gliosis surrounding prion deposition. The cause and effect of these cellular responses are still unclear. Here we investigate the impact of innate immune responses on prion replication using *in vitro* cell culture models.

Materials and Methods: Hamster-adapted transmissible mink encephalopathy prions, hyper (HY) and drowsy (DY) strains, were assayed for accumulation of pathogenic prion protein (PrP^{Sc}) in primary glial cultures derived from 8-day-old hamster pups.

Results: The kinetics of PrP^{Sc} accumulation largely depended on prion strain and brain regions from where glial cells originated. Glial cells derived from the cerebellum were susceptible to HY, but resistant to DY strain as determined by western blot analysis, immunocytochemistry, and animal bioassay. Glial cells from the cerebral cortex were, however, refractory to both strains. PrP^{Sc} accumulation was affected by innate immune modulators. Priming glial cells with a toll-like receptor (TLR) ligand, lipopolysaccharide, decreased prion replication, whereas pre-treatment with a synthetic glucocorticoid, dexamethasone, that inhibits innate immunity increased susceptibility to DY infection.

Conclusion: Our results suggest that neuroinflammation resulting from prion infection is a response to resolve and/or prevent prion propagation in the brain. It implies a therapeutic potential of innate immune modulation through TLR signaling in the early stages of prion disease.

Funded by: Judd Aiken and Debbie McKenzie

Grants: NSERC Discovery, Alberta Prion Research Institute, and Genome Canada.

Theme:

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Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
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Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	X

Title: Blood-based Nano-QuIC: Accelerated and Inhibitor-resistant Detection of Misfolded α-synuclein

Authors: Peter R. Christenson[1,2], Hyeonjeong Jeong[1], Manci Li[2,3], Hyerim Ahn[1], Danni Li[4],

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Aims: A hallmark of synucleinopathiesα-synucleinopathies (e.g. Parkinson's disease, dementia with Lewy bodies (DLB), and multiple system atrophy) is the misfolding and aggregation of alpha synuclein in tissues and biological fluids. Real-time quaking-induced conversion (RT-QuIC), a protein-based seeded-amplification assay, provides extremely sensitive detection of disease associated misfolded proteins yet is currently limited to invasive sample types such as cerebrospinal fluid testing for clinical use. More accessible sample types, such as blood, contain inhibitors that interfere with the RT-QuIC Assay. In this study we aim to show that silica nanoparticles can enhance RT-QuIC's utility in detecting misfolded alpha synuclein in human plasma and bovine whole blood.

Materials and Methods: We spiked human plasma and lysed whole blood bovine samples with misfolded alpha synuclein down to concentrations of 90 pg/ml. These samples were diluted in SDS detergent and added to QuIC reactions containing 50 nm silica nanoparticles (Nano-QuIC) and reactions with no silica nanoparticles (RT-QuIC). Samples were shaken and incubated 48 hrs at 48℃. Thioflavin T fluorescence readings were taken every 45 minutes.

Results: In spiked human plasma samples, Nano-QuIC showed 100 times improvement in sensitivity and while doubling the speed of the reaction. For spiked lysed whole bovine blood, Nano-QuIC detected concentrations of misfolded alpha synuclein down to 90 pg/ml while RT-QuIC failed to have any detection due to the presence of strong inhibitors. Crucially, no false positives were observed in human plasma or lysed whole bovine blood with these 50 nm silica nanoparticles, demonstrating great potential for diagnostic application of Nano-QuIC in detecting misfolded proteins.

Conclusions: In this study, we show that Nanoparticle enhanced RT-QuIC (Nano-QuIC) can greatly accelerate the speed and sensitivity of detection of misfolded alpha synuclein spiked into extremely complex samples such as human plasma and whole lysed blood from bovine compared to RT-QuIC.

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Theme	(X)
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Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	X
Therapeutic approaches for prion and prion-like diseases	

Ultrafast Amplification of Prions via Microfluidic Quaking Induced Conversion (Micro-QuIC)

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Aims: Protein misfolding diseases, encompassing prion diseases like CJD and animal diseases such as CWD, pose grave neurodegenerative threats to humans and animals alike. The enduring infectious nature of misfolded proteins and their potential to cross species barriers are significant concerns. This research focuses on CWD, a model for these diseases, with global spread in regions including North America, Scandinavia, and South Korea, elevating the risk of exposure. We explore acoustofluidic micromixers as a promising solution to enable homogeneous mixing of reagents in a high-Reynolds-number regime. In parallel, conventional QuIC assays leverage protein amplification and fluorescent signaling for the accurate detection of misfolded proteins, especially misfolded prion proteins. We combine acoustofluidic micromixers and QuIC technologies to create the Microfluidic Quaking Induced Conversion (Micro-QuIC™) assay, a field-deployable method for detecting CWD in under 3 hours.

Material and Methods: The Micro-QuIC™ device utilizes a thin glass coverslip and a PDMS channel equipped with lateral cavity structures that trap air cavities upon reagent injection. These liquid-air interfaces serve as oscillating membranes, responding to transducer vibrations and inducing acoustic streaming to agitate the samples. Tissue samples are mixed with a master mix containing recombinant HaPrP and then amplified by applying an input bias of 10 Vpp at 4.6 kHz. Amplification progress was monitored under fluorescent microscopy at 30-minute intervals. To showcase the potential for field-portable PrP^{CWD} diagnostics, we amplified positive and negative white-tailed deer lymphoid tissues using Micro-QuIC™ and subsequently assessed with gold-nanoparticle-based colorimetric detection (MN-QuIC).

Results: Micro-QuIC™ achieved successful amplification of PrP^{CWD}-spiked samples within 3 hours, with no measurable changes observed in negative samples. This represents a remarkable 16-fold reduction in amplification time compared to conventional RT-QuIC. In lymphoid tissue testing, Micro-QuIC™ accurately identified all 5 CWD-positive tissues and all 5 CWD-negative tissues, using naked eye. The results from RT-QuIC matched those of Micro-QuIC™ with 100% consistency.

Conclusions: By harnessing acoustofluidic technology in conjunction with the established RT-QuIC assay, we have substantially cut down the amplification time from 48 hours to a mere 3 hours. The resulting

Micro-QuIC device stands out for its simplicity, automation, cost-effectiveness, and portability, setting the stage for an integrated on-chip amplification toolkit. This innovative approach holds immense potential for on-the-spot diagnosis of protein misfolding diseases, with far-reaching benefits for both global health and wildlife management initiatives.

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Evolution of Nor98/ Atypical scrapie by iterative propagation in a homologous ovine PrP^C context

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Aims:
Nor98/ Atypical scrapie (AS) is a prion disease that causes sporadic cases in sheep and goats. Previous studies have shown that the transmission of AS to other species led to the emergence of new prion strains. In the bovine and porcine PrP, there has been reported the emergence of classical BSE prions (Huor et al., 2019, Espinosa et al., 2009, Marin et. al., 2021) and in the bank vole M109I-PrP context, a classical scrapie-like prion strain emerges (Pirisinu et al., 2022).
In this study, we analysed the possible evolution of the AS prion within the same specie by modelling the transmission in a homologous ovine PrP context.

Materials and Methods:
A panel of AS isolates with different genotypes and geographical origins both from sheep and goats were inoculated in the wild-type transgenic mice model (ARQ-PrP, Aguilar-Calvo et al., 2014).

Results:
The isolates infect the ovine ARQ-PrP mice with homogeneous survival time and a complete attack rate. For several AS isolates the transmission led to the emergence of 19kDa (with BSE-like characteristics), 21kDa or atypical prions and mixtures of these agents.

Conclusions:
Iterative subpassages of AS isolates into transgenic mice carrying ovine PrP showed an emergence of classical prions during *in vivo* propagation. This could be caused by the coexistence of strains in the isolate or the evolution of the AS through propagation in the ovine PrP.
These results allow us to hypothesize whether atypical prions might be the origin of prion diversity, where atypical prions tend to acquire classical forms. These results are relevant to control the exposure of farmed animals and humans to AS.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	(X)
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Effect of neuroinvasion on strain property maintenance for two α-synuclein prion strains

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Aims: Synucleinopathy patients exhibit heterogeneity in clinical presentation, neuropathology, and disease pathogenesis, which complicates clear antemortem diagnoses and therapeutic development. For example, patients can initially present with either a central autonomic dysfunction, or a peripheral dysfunction that later converts to a central disease. While exposure route is well-known to influence pathogenesis of classic prion diseases, it is unknown if or how peripheral exposure alters disease pathogenesis across the spectrum of α-synuclein prion strains. Moreover, it is unknown if neuroinvasion relies on the same neuroanatomical pathway from the periphery for all strains, or if strain-specific routes are involved. Here we test the hypothesis that, unlike some PrP strains, two α-synuclein strains will maintain their strain properties during neuroinvasion.

Materials and Methods: To investigate the strain-specific effects of exposure route on disease pathogenesis, we inoculated either 20 µL of mouse-passaged MSA (multiple system atrophy) brain homogenate or recombinant A53T preformed fibrils (PFFs) into 9- to 10-week-old TgM83$^{+/-}$ mice and compared the onset of neurological disease between sciatic nerve (sc.n.) and intracranial (i.c.) injections. Brains dissected from terminal animals were bisected down the midline and collected half frozen and half fixed for subsequent analyses. Spinal columns of sc.n.-injected mice were also collected and fixed for immunohistochemistry. Homogenates from the frozen half-brains were tested for infectivity in a panel of α-syn140-YFP biosensor cell lines expressing various mutations to assess biological activity.

Results: Mice injected i.c. with MSA developed clinical signs 118 ± 10 days postinoculation (dpi); incubation time was extended to 210 ± 56 dpi following sc.n. injection. By comparison, mice injected with A53T PFFs developed clinical disease 88 ± 18 dpi when injected i.c., versus 204 ± 42 dpi when injected in the sc.n. Notably, incubation period was extended by ~50% following sc.n. injection, regardless of strain. A-synuclein prions isolated from frozen brain tissue were similarly infective in the α-syn140*A53T-YFP biosensor cells, regardless of strain, suggesting α-synuclein prion content is consistent in terminal mice. However, strain-specific differences were observed across a panel of cell lines, indicating distinct biological activities between the two strains. This is consistent with the observation that the mouse-passaged MSA strain was more sensitive to proteinase K digestion, but more resistant to guanidine denaturation, compared to the passaged A53T PFFs. Despite these differences, mice injected with either strain developed similar phospho-synuclein pathology throughout the hindbrain, regardless of exposure route.

Conclusions: As anticipated, A53T PFFs transmitted disease to TgM83$^{+/-}$ mice with a shorter incubation period than MSA prions, regardless of injection route. Consistent with this finding, in i.c.-injected mice, the two strains induced α-synuclein prion formation with distinct biochemical and biological activities. Despite these clear strain differences, the distribution of neuropathological lesions was similar, as were the observed clinical signs. MSA prions retained their strain properties following transmission to TgM83$^{+/-}$ mice, demonstrating replication with high fidelity occurs regardless of exposure route. Analysis of sc.n. A53T PFF-inoculated mice is ongoing, but initial data indicate strain selection occurred in i.c.-injected animals. Our findings suggest that location of disease origin does not alter disease pathogenesis for MSA prions, which has important implications for treating patients with a peripheral synucleinopathy that converts into MSA.

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Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

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Experimental Oronasal Inoculation of the Chronic Wasting Disease Agent into White Tailed Deer

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Aims: The purpose of this experiment was to determine whether white-tailed deer (WTD) are susceptible to inoculation of chronic wasting disease (CWD) via oronasal exposure.
Materials and methods: Six male, neutered WTD were oronasally inoculated with brainstem material (10% w/v) from a CWD-positive wild-type WTD. The genotypes of five inoculated deer were Q95/G96 (wild-type). One inoculated deer was homozygous S at codon 96 (96SS).
Cervidized (Tg12; M132 elk PrP) mice were inoculated with 1% w/v brainstem homogenate from either a 96GG WTD (n=10) or the 96SS WTD (n=10).
Results: All deer developed characteristic clinical signs of CWD including weight loss, regurgitation, and ataxia. The 96SS individual had a prolonged disease course and incubation period compared to the other deer. Western blots of the brainstem on all deer yielded similar molecular profiles. All deer had widespread lymphoid distribution of PrP^{CWD} and neuropathologic lesions associated with transmissible spongiform encephalopathies. Both groups of mice had a 100% attack rate and developed clinical signs, including loss of body condition, ataxia, and loss of righting reflex. Mice inoculated with material from the 96SS deer had a significantly shorter incubation period than mice inoculated with material from 96GG deer (Welch two sample T-test, $P<0.05$). Serial dilutions of each inocula suggests that differences in incubation period were not due to a greater concentration of PrP^{CWD} in the 96SS inoculum. Molecular profiles from western blot of brain homogenates from mice appeared similar regardless of inoculum and appear similar to those of deer used for inoculum.
Conclusions: This study characterizes the lesions and clinical course of CWD in WTD inoculated in a similar manner to natural conditions. It supports previous findings that 96SS deer have a prolonged disease course. Further, it describes a first pass of inoculum from a 96SS deer in cervidized mice which shortened the incubation period.

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Animal prion diseases	X
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Conference theme: Animal prion diseases

Atypical BSE cases in Ireland: neurological signs, brain histopathology and tissue distribution of PrPres

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Aims:

In Ireland, six atypical BSE cases, five H-type (H-1 to -5) and one L-type, have been confirmed up to May 2023.

Herein, the neurological characteristics, brain histopathology, topographical distribution, and signal intensity of PrP^{res} are described.

Material and Methods:

All cases were identified through active surveillance. Clinical history was retrieved from the Department of Agriculture, Food and the Marine archives. Whole brains/brainstems of H-type animals, and the L-type, and selected peripheral tissues of L-type were further studied by histopathology, immunohistochemistry (IHC - MAb F89) and immunoblotting (APHA BioRad TeSeE Hybrid). Investigations on PrP^{res} distribution on the H-5 are in progress.

Results:

All animals were beef-breed females, aged between 11 – 18 years-old. They had vague clinical histories of depression, inappetence, incoordination, and recumbency, lasting 2-4 days. In the L-type and in H-5 intermittent signs lasted 2 and 6 weeks, respectively. H-2 was a healthy slaughtered animal.

Among the suitable obices for histopathology (H-1, -2 and -5), and the whole brain of H-5, vacuolation was only detected in H-5. Positive immunostaining was detected at the obex for H-1, in medulla, thalamus, cerebellum for H-2, and at all levels of the brain for H-3 and H-5. In the fallen H-type cases, immunoblot and Idexx EIA were consistently strong in all brain levels. In the healthy slaughter animal, PrP^{res} levels were lower in cerebellum and cerebral cortex.

L-type showed inconclusive histopathological changes at obex, whilst neuropil vacuolation was most marked in thalamus and midbrain. PrP^{res} was detected by IHC, immunoblotting and Idexx EIA at all levels of the brain and spinal cord, and immunoblotting only in the optic nerve and retina.

Conclusions:

Clinical courses were short and non-specific. PrP^{res} intensity in all cases were generally high at all levels of the brain tested including the obex, the official target area for BSE surveillance.

Acknowledgements: Colleagues in Regional Veterinary Laboratories for collecting the brain material. Colleagues in TSE Division and DVOs for clinical information on cases.

Determination of Ubiquitin C- Hydrolase L 1 (UCH-L1) in the body fluids of sCJD patients

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Aims

Fluid biomarkers are quite important for the differential diagnosis of neurodegenerative diseases. CSF is an essential source of biomarkers to help the diagnosis of sporadic CJD that does not have any genetic background. Some markers are validated in CSF, but the availability of blood biomarkers is important to have a less invasive method for diagnosis. In this study, we investigated the level of Ubiquitin C-Hydrolase L 1 (UCH-L1) which has been associated with PrP^{Sc} aggregates.

Material & Methods

We used CSF and serum samples from sCJD and aged- and gender-matched non-neurodegenerative controls (NNC). We determined the level of UCH-L1 protein in body fluids using commercially available ultrasensitive SIMOA assays.

Results

Our analysis shows that the level of UCH-L1 in CSF is significantly higher in sCJD patients than in the control group ($p<0.0001$). To determine the diagnostic accuracy, we performed a ROC curve analysis. It showed a high AUC value to differentiate sCJD from NNC in CSF. Afterward, we also checked the regulation in serum samples from both groups. Our results showed that sCJD has a significant elevation in serum which is in line with CSF. ROC curve analysis also showed high accuracy to distinguish between sCJD and NNC.

Conclusions

To conclude, our work showed that UCH-L1 has a good potential for the discrimination of sCJD from NNC both in CSF and blood. It is also detectable in blood which is quite advantageous. After the validation of the marker, it has a good potential to be used in diagnostics.

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Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	x
Therapeutic approaches for prion and prion-like diseases	

MAPT and _SNCA_ gene expression in cells with protein cytopathologies in Tau- and Synuclein-related neurodegenerative diseases

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Aim: Accumulation of misfolded tau and α-synuclein contribute to neurodegeneration in progressive supranuclear palsy (PSP) and Lewy body disease (LBD), respectively. It is not known whether accumulation of phosphorylated tau or α-synuclein inclusions are associated with altered *MAPT* and SNCA expression in neurons or glia. Therefore, we examined the relation of *MAPT* and *SNCA* transcripts to tau and α-synuclein cytopathologies.

Materials and Methods: RNAseq was used to quantify *MAPT* and *SNCA* expression across different cell types in controls (n= 3 for *MAPT* and 5 for *SNCA*), PSP (n=3) and LBD (n=5). The anatomical distribution of *MAPT* and *SNCA* transcripts in different cell types and brain regions was mapped using RNAscope, which was combined with p-tau (AT8) and α-synuclein (5G4) immunofluorescence.

Results: *MAPT* gene expression was found in neurons, oligodendroglia and astrocytes. Volume density of *MAPT* transcripts varied between brain regions and within each cell type suggesting distinct sub-populations of cells within neurons, oligodendroglia and astrocytes. In PSP, the presence of *MAPT* transcripts was confirmed in cells that contained phosphorylated tau inclusions, including neurofibrillary tangles, oligodendroglial coiled bodies, and tufted astrocytes. The volume density of *SNCA* transcripts in neurons with α-synuclein pre-aggregates was preserved and similar to neurons without α-synuclein deposits. However, a decrease of *SNCA* transcripts was observed in neurons with compact inclusions

Conclusions: Similar to neurons, oligodendroglia and astrocytes contain *MAPT* transcripts indicating that glia have *MAPT* protein expression that can potentially be phosphorylated and fibrillized into pathological inclusions. In both tau-and α-synuclein-related diseases *MAPT* and *SNCA* gene expression in protein-pathology-containing cells is preserved. A complete loss of disease-associated transcripts and related proteins is less likely as an early driver of the pathogenic process.

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Abstract title:

Neuron-Specific Expression of Zinc-Finger Repressor Mediates Widespread Prion Reduction in the Brain for the Potential Treatment of Prion Disease

Shih-Wei Chou, Kimberly Marlen, David Ojala, Giulia Cisbani, Finn Peters, Garrett Lew, Meredith Mortberg, Chiara Melis, Jing Hu, Michael Howard, Samantha Graffam, Kenney Lenz, Tyler Caron, Qi Yu, Sarah Hinkley, Alicia Goodwin, Mohad Mehrabian, Asa Hatami, Alaric Falcon, Marian Glynn, Kathleen Meyer, Jason Fontenot, Amy M Pooler, Eric Vallabh Minikel, Sonia M Vallabh, Bryan Zeitler

Aims:

Misfolding of cellular prion protein, PrP, causes rapidly progressing and invariably fatal prion disease. We are investigating epigenetic regulation of the prion gene (*PRNP*) using zinc-finger-repressor (ZF-R) approach as a potential therapeutic strategy to achieve widespread, rapid, and sustained reduction of brain PrP. Cellular PrP is ubiquitously expressed and *PRNP* transcripts are abundant in neurons and glia cells. Several lines of evidence suggest that neuronal PrP is necessary and sufficient for neurotoxicity and disease progression. Our previous results showed substantial survival benefit in PrP^{Sc}-inoculated mice treated with a neuron-specific ZF-R at 60 and 122 days-post-inoculation (dpi).

Materials and Methods:

A ZF-R that represses *Prnp* expression >90% in mouse cortical neurons was paired with promoters that have known expression patterns: hSYN1 (neuronal), GfaABC1D (astrocytic), or CMV (ubiquitous). These promoter-ZF-R constructs were delivered to wildtype mice using a blood-brain-barrier (BBB) penetrating tool capsid (AAV.PHP.B). Prion mRNA and protein reduction were assessed in multiple brain regions. We also tested a potential clinically translatable BBB-penetrant AAV capsid in nonhuman primates.

Results:

Bulk RT-qPCR results showed 40-70% mouse prion mRNA reduction depending on the brain region and promoter tested, ranked as follows: hSYN1 ≥ CMV > GfaABC1D. Multiplexed RNAscope-immunohistochemistry and single-nucleus RNA sequencing demonstrated a strong negative correlation between ZF-R and *Prnp* expression for all promoters. The hSYN1 promoter was highly neuron-specific, the CMV promoter drove heterogeneous expression in neurons and astrocytes, and the GfaABC1D promoter drove weaker, non-specific expression in astrocytes and neurons. In nonhuman primates receiving intravenous dosing of a tool reagent hSYN1-ZF-R, we observed a similar biodistribution and repression pattern to the mouse experiments.

Conclusions:

Our results suggest that neuron-specific ZF-R expression has the potential to achieve sustained and widespread prion (mRNA/protein) reduction in mice and nonhuman primates, further supporting the development of a ZF-R-based genomic medicine for the treatment of prion disease.

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Grant number:

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Theme	x
Neuropathology of prion diseases	
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Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like disease	x

Amyloid from SARS-COV-2 Spike protein cross-seeds Human prion protein and Aβ

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Aims: SARS-CoV-2 infection and long COVID-19 are associated with several symptoms strikingly similar to blood coagulation and fibrinolytic disturbances [1] as well as neurologic and cardiac problems associated with amyloid disease. We have shown that SARS-CoV-2 spike protein (S-protein) is amyloidogenic [2]. Recombinant expressed full-length S-protein forms amyloid *in vitro* after cleavage by neutrophil elastase (NE) [2], a protease abundant at site of SARS-CoV-2 infection or mRNA vaccine injection [3]. S-protein-derived peptides have been suggested to accelerate neurodegenerative diseases [4]. Aβ levels in CSF from patients suffering neurological complications after COVID-19 infection are similar to what is detected in Alzheimer´s disease [5].

Does Spike-derived amyloid accelerate amyloid formation of proteins associated with neurodegenerative diseases?

Materials and Methods: Recombinant Aβ, Tau and Human prion protein (HuPrP) were *in vitro* subjected to seeding by Spike peptide amyloid fibrils as well as control seeds from other amyloidogenic proteins. Mouse inoculation experiments are ongoing.

Results: Seeding recombinant Aβ1-42 with S-protein amyloid accelerates the fibrillation rate. Amyloid formation of HuPrP *in vitro* is also accelerated by the presence of Spike peptide amyloids. Fibrils of S-sequence 601-620 was most efficient in seeding Aβ1-42, and S-sequence 532-551 most efficient in seeding HuPrP [6].

Conclusions: Our data propose a molecular mechanism for potential neurological manifestation of SARS-CoV-2, mediated by endoproteolysis of the Spike protein. The prospective of S-protein amyloidogenesis in SARS-CoV-2 Spike associated pathogenesis can be important in understanding the acute and long COVID-19. A recent review highlights a possible connection between other viral infections and increased risk of neurodegenerative disease later in life [7], serving as a reminder that amyloidogenic viral proteins may play a role in developing these devastating diseases later in life.

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Theme	
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Functional protein aggregation in yeast and mammalian systems	
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Therapeutic approaches for prion and prion-like diseases	

Discordant RT-QuIC results in siblings with genetic Creutzfeldt-Jakob disease linked to a rare five octapeptide repeats insertion in *PRNP* gene

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Aims: Real-Time Quaking-Induced Conversion (RT-QuIC) assay is recognized as sensitive and specific diagnostic tool for sporadic CJD. Its performance in detection of different forms of genetic CJD (gCJD) is variable. Here, we studied the presence of prion converting activity in *post-mortem* tissues of blood relatives, sister (54 years) and brother (65 years) with gCJD caused by a rare five octapeptide repeats insertion (5-OPRI). Both patients presented with progressive cerebellar ataxia, cognitive decline, and a slow clinical course lasting more than a decade.

Materials and Methods: A 120 bp insertion of five octapeptide repeats: R1-R2-R2-R3-R4-[R2-R2-R3-R3-R4] was confirmed by *PRNP* sequence analysis. We utilized RT-QuIC using recombinant Syrian hamster (rHaPrP90-231) or Bank vole (rBVPrP23-230) prion protein (PrP) to analyze brain homogenates (BH) from frontal lobe, ventricular cerebrospinal fluid (CSF) and skin tissue samples. To correlate the presence of prion converting activity with the presence of PrP deposits detected by immunohistochemistry (IHC) we analyzed archival formalin-fixed paraffin-embedded (FFPE) brain tissues.

Results: All samples of sister yielded clearly positive RT-QuIC signal except for skin samples which provided signal bellow the calculated threshold. Titration of 10% BH gave positive results up to $5x10^{-7}$ dilution using rSHaPrP90-231 and up to $5x10^{-8}$ using rBVPrP23-230 substrate. *Post-mortem* CSF was positive even when 10x diluted. Cerebellum FFPE 10% homogenate was positive up to 10^{-3} dilution. Strikingly, RT-

QuIC analysis of identical tissues of her brother provided negative results despite the evident presence of PrP deposits demonstrated by IHC.

Conclusion: We report here two siblings with a 5-OPRI genetic mutation, atypical disease course and dramatic difference in prion seeding activity present in their tissues. If this disparity was caused by the presence of different prion strains or by some other phenomenon remains to be elucidated.

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Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	X
Therapeutic approaches for prion and prion-like diseases	

Genetic Diversity of the Prion Protein Gene in German, Danish and Polish Cervids

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Aims: Chronic Wasting Disease (CWD) has recently emerged in Scandinavia in novel contagious and 'atypical' forms. Genetic variations within the Prion Protein Gene (*PRNP*) modulate susceptibility of cervids to CWD. For future European risk assessment, we aim to define *PRNP* genotypes in German and Danish red deer *(Cervus elaphus)*, German roe *(Capreolus capreolus)* and sika deer *(Cervus nippon)* and Polish moose *(Alces alces)*.

Materials and Methods: In collaboration with hunters, foresters and pathologists, we collected samples during the hunting season 2021/22 throughout Germany and from the Danish Frøslev Plantage. The Polish samples were collected from 2018 to 2020 for CWD monitoring. We sequenced the open reading frame on exon three of the *PRNP*.

Results: A representative selection of submitted samples 297/2225 roe, 278/843 red and 40/40 sika deer from Germany, 25/25 Danish red deer and 81/107 Polish moose were genotyped. All roe deer (297/2225) are of wildtype $T_{98}P_{168}Q_{226}$/TPQ with five animals (5/297) showing a silent mutation (SM) at codon 42. However, genetic variability was seen in German and Danish red deer. Two non-synonymous polymorphisms at codons 98 and 226, one 24bp deletion and three SM at codons 21, 78 and 136 were detected. In total homozygote genotypes TPQ (50/278), TPE (116/278), APQ (9/278) and heterozygote genotypes TPQ/TPE (44/278), TPQ/APQ (20/278) and TPE/APQ (39/278) were detected. Genotypes TPQ (36/40), TPQ/TPE (3/40) and TPE/APQ (1/40) were seen in sika deer as well as SMs at codon 133 (4/40) and 136 (4/40), the former being sika-specific. 81/81 of the Polish moose samples were homozygous for TPQ with one SM at codon 20 in 11/81 animals.

Conclusions: How the genotypes influence CWD-susceptibility remains to be tested. Nevertheless, these results complement the knowledge of European cervid genotypes and contribute to the adjustment of the CWD surveillance and control measures.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	X
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Detection of prions in soils contaminated by multiple routes

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Aims: Free-ranging animals afflicted with transmissible spongiform encephalopathies frequently shed infectious prions into the broader environment. The quintessential example is chronic wasting disease, the TSE of cervids. Over the course of the disease, an infected animal will shed infectious prions in blood, urine, saliva, and feces. Upon death, the total prion load interred in the animal's tissues will be deposited wherever the animal falls. This contamination creates substantial risk to naïve animals, and likely contributes to disease spread. Identification and quantification of prions at contamination hotspots is essential for any attempt at mitigation of environmental transmission.
Materials and Methods: Surfactant extraction of soils followed by precipitation yields a sample that is amenable to analysis by real-time quaking induced conversion. However, differences in extraction yield are apparent depending on the properties of the matrix from which the prions are being extracted, principally soil clay content.
Results: We are able to detect prion seeding activity at multiple types of environmental hotspots, including carcass sites, contaminated captive facilities, and scrapes (i.e. urine and saliva). Differences in relative prion concentration vary depending on the nature and source of the contamination. Additionally, we have determined that prion seeding activity is retained for at least fifteen years at a contaminated site following attempted remediation.
Conclusions: Detection of prions in the environment is of the utmost importance for controlling chronic wasting disease spread. Here, we have demonstrated a viable method for detection of prions in complex environmental matrices. However, it is quite likely that this method underestimates the total infectious prion load in a contaminated sample, due to incomplete recovery of infectious prions. Further refinements are necessary for accurate quantification of prions in such samples, and to account for the intrinsic heterogeneities found in the broader environment.

Funded by: Wisconsin Department of Natural Resources

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
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Animal prion diseases	X
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Tau aggregate conformations are readily modified by disease-associated mutations

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Aims: Frontotemporal Lobar Degeneration Tau (FTLD-tau) can be associated with a large number of mutations in the MAPT gene encoding tau protein. Over 40 identified mutations are classified as missense mutations that alter the primary amino acid sequence of tau. Tau missense mutations have been widely used in experimental models for understanding tau pathobiology. One key aspect of tau biochemistry that may influence its pathogenic properties but is understudied is the ability of missense mutations to directly alter tau aggregate conformation. We set out to define which specific tau missense mutations drive the formation of distinct tau aggregate structures.

Materials and Methods: We created a high-throughput biochemical platform for purifying and assaying recombinant WT 0N4R tau and 36 missense mutants, in parallel. Purified tau was induced to aggregate *in vitro*. Trypsin digest assays were used to identify packed core regions of tau aggregates. Thioflavin T assays were used to monitor *in vitro* aggregation kinetics.

Results: Analysis of the protease-resistance cores from *de novo* formed tau aggregates revealed that a large subset of mutations generated aggregate conformations that were distinct from WT tau (13 discrete conformations identified). Multiple mutations shared the same non-WT aggregate conformation fingerprint. Parallel analysis of aggregation kinetics showed no correlation between the kinetic properties of mutants and their ability to form a given aggregate structure. We found that our observed effects of mutations on aggregate structure could not readily be predicted by current Cryo-EM structure information.

Conclusions: Our comprehensive analysis of 36 tau missense mutants, revealed that a subset of tau mutations encode for the formation of aggregate conformations distinct from WT, identifying a potential new mechanism by which these mutations promote disease. Several of the structure-altering mutations (e.g. P301L, P301S, V337M) are commonly used in experimental research models. Given the wealth of evidence that aggregate conformations can modulate key features of prion and prion-like diseases, we believe that our study highlights the need to further explore the relationship between tau mutation, aggregate structure, and downstream pathogenic mechanisms.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	X
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion- like diseases	X
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Spontaneous emergence of infectious protease-resistant prions in knock-in mouse models of inherited prion diseases

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Aims: Most prion diseases arise spontaneously without any infection from an exogenous source. However, the mechanism that governs the initial misfolding events of PrP^C in the brain remains unclear as recreating these experimentally has proven challenging. Recent transgenic mouse models utilizing the unique misfolding properties of bank vole prion protein (BVPrP) have been able to recapitulate the emergence of prions *in vivo*, but whether the same can occur at physiological levels of protein expression remains undetermined. In this study, we aimed to model spontaneous prion formation in a more refined paradigm by expressing BVPrP at physiological levels in knock-in mice.

Materials and Methods: We created a series of knock-in mice expressing wild-type or mutant BVPrP with isoleucine at codon 109. Two mutations were investigated: D178N and E200K, which cause Fatal Familial Insomnia and familial Creutzfeldt-Jakob disease, respectively. The brains of healthy and spontaneously sick mice were analyzed for the presence of protease-resistant PrP and disease-specific neuropathology. Further, the generation of authentic prions was confirmed by intracerebrally inoculating brain extracts from spontaneously sick mutant BVPrP knock-in mice into wild-type BVPrP(I109) mice, which do not themselves get sick.

Results: Knock-in mice expressing wild-type BVPrP(I109) remained free of any neurological illness for their entire lifespan. In contrast, knock-in mice expressing either D178N- or E200K-mutant BVPrP(I109) exhibited progressive signs of neurological illness, prion disease-specific neuropathology, and emergence of protease-resistant PrP species within the brain. Moreover, brain extracts from spontaneously sick mutant BVPrP(I109) knock-in mice successfully transmitted disease to wild-type BVPrP(I109) knock-in mice, confirming the generation of infectious prions.

Conclusions: Our study demonstrates that knock-in mice expressing mutant BVPrP(I109) at physiological levels recapitulate key hallmarks of human prion disease, including the spontaneous generation of *bona fide* prions. These knock-in mice will help delineate the earliest events involved in genetic prion diseases.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	(X)
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Prion 2023 abstract title: ***In vitro*** **amplification activity of abnormal prion protein aggregates recovered from archived Appendix II and III asymptomatic cases using highly sensitive Protein Misfolding Cyclic Amplification**

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Aim: The detection of positive abnormal prion protein (PrP) aggregates during the retrospective immunohistochemical surveys of fixed, paraffin-embedded (FFPE) appendectomy samples (informally known as Appendix studies), in specimens originating from the pre- and post-bovine spongiform encephalopathy (BSE) exposure eras in the UK, questioned their connection with the recognised variant Creutzfeldt-Jakob disease (vCJD) prion strain. We recently reported a robust method able to recover abnormal PrP aggregates from FFPE vCJD brain and appendix tissue sections, and provided evidence that the product of the serial highly sensitive Protein Misfolding Cyclic Amplification (hsPMCA) conserves the biochemical profile of the vCJD strain. The aim of this study is to apply this method to retrieve abnormal PrP aggregates from immunohistochemistry-positive Appendix studies II and III asymptomatic cases and investigate their relationship with the vCJD prion strain.

Material and Methods: Archived FFPE samples derived from the Appendix studies II and III were deparaffinised with heated water at 85°C followed by homogenisation using a sterile plastic pestle combined with ultrasonication, in order to retrieve PrP aggregates. The amplification potential of the recovered aggregates was evaluated by hsPMCA, and prion strain biochemical properties were assessed by Western blotting.

Results: hsPMCA successfully amplified PrP aggregates from a subset of immunohistochemistry-positive samples originating from Appendix II and III surveys. The

resulting biochemical profile was consistent with the characteristic "2B" vCJD signature in all hsPMCA-positive cases.

Conclusion: The amplification and further molecular typing of the FFPE extracted material from positive appendices from archived Appendix II and III asymptomatic samples produced a molecular profile consistent with vCJD. A further comprehensive analysis of the positive hsPMCA-amplified cases, in addition to complementary animal transmission studies is currently ongoing.

Words 274/300

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Theme	
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	

Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	**(X)**
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Prion 2023 abstract title:
Prion disease features in Japan based on the national surveillance from 1999 to 2023

Authors: Tadashi Tsukamoto 1), Koki Kosami 2), Ryuusuke Ae 2), Tsuyoshi Hamaguchi 3), Nobuo Sanjo 4), Katsuya Satoh 5), Tetsuyuki Kitamoto 6), Masaki Takao 7), Masahito Yamada 8), and Hidehiro Mizusawa 1) and Prion Diseases Surveillance Committee in Japan
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8 Division of Neurology, Department of Internal Medicine, Kudanzaka Hospital, Tokyo, Japan.

Aim: To elucidate epidemiological features of prion disease (PrD) in Japan.

Methods: Nationwide surveillance has been conducted by Prion Disease Surveillance Committee in Japan since 1999. The committee was composed of 10 district members and specialists in epidemiology, neurosurgery, genetics, EEG, imaging, CSF biomarkers, and genetic counseling. Clinical information on suspected PrD cases was compiled and reviewed in the committee twice yearly, resulting in diagnoses of PrD and non-PrD.

Results: Information of 6936 suspected PrD patients was obtained and until February 2023 there were 4714 PrD cases [sporadic CJD 3578 cases, variant CJD 1, dura matter-associated CJD (dCJD) 93, and genetic PrD 1024 (genetic CJD 842, 174 Gerstmann-Sträussler-Scheinker disease 174, fatal familial insomnia 8)]. The annual incidence increased from 0.7 to 2.3 per million between 1999 and 2015. Genetic analysis showed a high ratio of methionine/methionine (95%) at codon 129 in sporadic CJD. Effects of polymorphisms at codon 129 and 219 to various PrDs were investigated.

Conclusions: Our surveillance confirmed the increase of PrD incidence and characteristic features of PrDs in Japan. Continuous surveillance of PrDs is very important and has contributed greatly to various types of research to overcome PrDs.

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Theme	(X)
Neuropathology of prion diseases	
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Protein structure, function, conversion, and dysfunction	
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Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	X

Efavirenz, an FDA approved medication prolonged the survival of CJD-infected tg650 mice

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Aims: Recently, we found that Efavirenz (EFV), an FDA approved anti-HIV medication which activates the brain cholesterol metabolizing enzyme (CYP46A1), reduced infectious prion protein (PrP^{Sc}) levels and significantly increased the survial of RML-infected C57BL/6J mice. We aimed to extend our approach towards preclinical trials using transgenic tg650 mice overexpressing human PrP^{C} infected with sporadic Creutzfeldt-Jakob disease (sCJD) brain homogenates to test EFV treatment and CYP46A1 overexpression on pathogenesis.
Materials & Methods: After intracerebral inoculation (20 µl 1% sCJD brain homogenates) of tg650 mice, five groups (n=15/group) were used *(1)* sCJD-infected tg650 mice, *(2)* sCJD-infected tg650 mice+CYP46A1-AAV at 30 dpi, *(3)* sCJD-infected tg650 mice+GFP-AAV at 30 dpi, *(4)* sCJD-nfected tg650 mice+EFV at 30 dpi and *(5)* sCJD-infected tg650 mice+EFV at 130 dpi. CYP46A1-AAV and GFP-AAV recombinant viral particles were administered via retro-orbital injection. EFV (1.68 mg/L) was supplied in drinking water and continued until the experimental endpoint. Signs of prion disease appeared at 176 dpi, and we euthanized 5 mice/groups for analyses of PrP^{Sc} level. The remaining mice were euthanized at terminal prion disease. Differences in survival between control and treated groups were analyzed by Kaplan–Meier plot and log rank test.
Results: Our survial data show that EFV treatment starting at 30 dpi significantly (***$p<0.001$) increased (+17 days) the survial of mice as compared to the non-treated mice. Similarly, EFV treatment starting at 130 dpi also significantly (***$p<0.001$) extended (+23 days) survival of mice as compared to non-treated mice. Unexpectedly, CYP46A1 overexpression did not increase the survival of mice. This indicates that a single administration of CYP46A1 overexpressor was not sufficient to restore CYP46A1 activity. These results will be validated with biochemical and morphological assessments.
Conclusions: EFV is a safe and effective medication at low dosage in a model of sCJD, suggesting a valuable therapeutic to treat human prion diseases.

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Theme	
Neuropathology of prion diseases	
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Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	(X)

Targeting cellular prion protein via peptide aptamers to treat prion and prion like diseases

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Aims: Prions and prion like diseases (Alzheimer's disease (AD)) are incurable protein misfolding neurodegenerative diseases. The cellular prion protein (PrP^{C}) plays a vital role in both diseases. PrP^{C} acts as a substrate for replication of infectious prion (PrP^{Sc}) and as a high affinity receptor for amyloid beta oligomers (AβO). AβO is a neurotoxic species of Aβ which accelerates AD pathologies. We developed peptide aptamers (PAs) targeting PrP^{C}. PAs consist of a combinatorial peptide moiety inserted into a protein scaffold, here the *E. coli* thioredoxin A (TrxA). The aim of our research is to use PAs to treat AD and prion diseases.
Materials & Methods: Recombinant PAs were used to treat prion infected neuronal cells and primary neurons to determine effects on prion replication and AbO toxicity. Following *in vitro* experiments, 5XFAD mice were treated with PA8 and scaffold protein TrxA (as a control) at a 14.4 ug/day dosage for 12 weeks by intraventricular infusion using Alzet® osmotic pumps. Behavioral and biochemical/immunohistological experiments were performed.
Results: Our *in vitro* results indicate that PA treatment inhibits the propagation of PrP^{Sc}. We also found that PA treatment prevents the interaction of AβO with PrP^{C} and reduces AβO-induced neurotoxicity in N2a cells and primary hippocampal neurons. For the first time, we observed that treatment with PA8 improves memory functions of 5XFAD mice as compared to Trx-treated 5XFAD mice. We found that PA8 treatment significantly reduces AβO pathologies in the brain tissue of 5XFAD mice. Interestingly, PA8 significantly reduces AβO-PrP interaction and its downstream signaling such as phosphorylation of Fyn kinase, reactive gliosis and neurodegeneration in 5XFAD mice.
Conclusions: Overall, our results suggest that PA8 would be a novel therapeutic tool to treat prion and prion like diseases.

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Theme	
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Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	(X)

Brain region-dependent distinct neuronal cell deaths in prion-infected mice

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Aims: Neuronal loss is one of the hallmarks of animals affected with prions; however, the mechanism remains unclear. We attempted to elucidate the mechanisms of neuronal cell death induced by prions.
Materials and Methods: Two mouse-adapted prions, 22L and Obihiro strain, were inoculated into ICR mice intracranially. The mice were sacrificed at 60, 90, 120, 145 days post inoculation (dpi) and brains were collected for histopathological and biochemical analysis. The involvement of four well-known regulated cell deaths, apoptosis, necroptosis, pyroptosis, and ferroptosis, were analyzed.
Results: We have reported that the decrease in neurons and induction of ATF3, a stress-inducible transcriptional regulator, become appeared around 90 dpi in neurons at lateral dorsal nuclei (LD) of the thalamus of mice infected with prions (Shimakura et al, prion 2020). No TUNEL-positive or cleaved caspase-3-positive cells were observed in the LD, suggesting that apoptosis is not the major process of neuronal death in the LD. Additionally, neither pyroptosis assessed by anti-cleaved GSDMD antibody nor necroptosis assessed by anti-phosphorylated MLKL antibody could be detected in this region. On the contrary, immunohistochemical analysis revealed the accumulation of 4-HNE, a marker for lipid peroxidation, and the decrease in GPx4, selenoenzyme that reduces membrane phospholipid hydroperoxides, in the neurons at the LD region. RNAscope analysis confirmed the expression of *Chac1*, the glutathione degrading enzyme gene, in the ATF3-positive neurons and 4-HNE level in ATF3-positive neurons appeared to higher than that of ATF3-negative neurons. Given that these results suggest the ferroptosis in the thalamic neurons. On the other hand, interestingly, TUNEL-positive neurons were observed in granular cell layer of cerebellum of prion-infected mice at the terminal stage of the disease.
Conclusions: These results show that at least two distinct neuronal death processes, ferroptosis in the thalamus and apoptosis in the cerebellum, are induced in the brain of prion-infected mice.

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Theme: Neuropathology of prion diseases

Prion 2023 abstract title

An undiagnosed case of sCJD-129MV in cadavers for forensic anatomy using RT-QuIC

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Aim:

The incident rate of prion diseases has been reported one or two per million people. We have reported that an undiagnosed case for prion diseases was found in 75 donated cadavers for anatomical practice in medical school. To elucidate rate of undiagnosed or preclinical carrier CJD, we conducted the prion screening tests for cadavers in forensic anatomy using real time quaking induced conversion (RT-QuIC) method.

Materials and Methods:

Cases were selected at random from the tissue stocks of nine different laboratories of forensic anatomy in Japan and brain necropsy samples were collected from both frontal and temporal cortex. Frozen samples were sent to our facility and one pieces of cortex from each different area was homogenized in our BSL3-laboratory for the test and the another was stocked at -80 ℃ until use for confirmation test. RT- QuIC was done with intermittent double orbital shaking at 1,000 rpm for 30 seconds followed by 30-seconds rest at 37 ℃ using Fluostar OMEGA (BMG Labtech). Human recombinant PrP (a.a. 23-231) was used as substrate and 10^{-6} g of samples were added to each well on first screening test.

Results:

Until now, we have screened 349 cases and found a RT-QuIC positive case. The case was 85yo women who has died with heart failure induced by heat hydration. Any neurological abnormality nor sign of dementia has not been observed before dead according to her family living near her home. Genetic analysis revealed she has heterozygous at codon 129 (MV) of *prnp gene*. On histopathological analysis, there

were no changes which indicate prion disease as spongiform change nor accumulation of abnormal PrP.
Conclusions: This cadaver could be a preclinical sporadic CJD-129MV with coordinate of only small amount of PrP^{Sc} accumulation and no histological change. We were not able to determine type 1 or 2 by Western blotting of PK-treated PrP. It is premature to discuss on frequency CJD in elderly population, and we are planning to continue this postmortem severance study, however, it would be important to think the possibility of undiagnosed CJD in forensic anatomy (and also in surgery).

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Grant number: H29-036

Theme	(X)
Neuropathology of prion diseases	(X)
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Engineering a knock-in mouse model to test human sequence-targeted therapeutics without the biohazard of human prions

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Aims: Animal models are an effective tool for testing therapeutic effect on various diseases; however, genetic medicines targeted to human sequences are rarely cross-reactive for endogenous mouse sequences. We aim to create a mouse model that would allow efficacy studies using human *PRNP* targeting compounds without the biohazard risk of human prions.

Materials and Methods: Animals were created using CRISPR/Cas9 gene editing. Brain PrP was quantified using an established in-house ELISA and *Prnp* RNA expression was quantified by qPCR. Animals were intracerebrally inoculated with RML prions to compare survival time and disease course. Animals were monitored for behavioral changes, weight loss, nest building and survival. Plasma was obtained monthly for serial NfL measurements.

Results: The new mouse model, termed ki7801, has all *Prnp* intronic, 3' and 5'UTR, signal peptide and GPI signal sequence replaced with human *PRNP* sequence, while the sequence coding for mature post-translationally modified PrP — codons 23-231 — is murine. Ki7801 animals showed no obvious phenotype and bred at the expected Mendelian ratio. No statistically significant difference in PrP expression was observed between ki7801 and wild-type animals. A pilot survival study in RML prion-inoculated animals is ongoing.

Conclusion: This model should permit testing of human drug candidates at a reduced biohazard level.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	X

Isolation and characterization of Stress granules from human brain

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#: Equal contribution

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Aims: Stress granules (SGs) are cytoplasmic membrane-less organelles composed of RNA and protein complexes that play a critical role in mRNA metabolism and translation. Emerging evidences suggest SGs' role in protein aggregation that is a hallmark of neurodegenerative disorders. In Alzheimer's disease (AD), pathological tau has been found to interact with RNA-binding proteins for example TIA-1, which may explain SGs' role in protein aggregation. In our study, we intend to isolate and analyze the composition of the endogenous stress/RNP granules from the human brain to understand its role in protein aggregation.

Methods: In this study, we analyzed 12 human brain samples (four in each group including AD, rpAD and healthy subjects). Brain samples from the frontal cortex were homogenized in an extraction buffer followed by differential centrifugation. Keeping in view the complexity of crude brain lysates, the enrichment of RNPs was performed by gradient fractionation. We identified the fractions enriched with TIA-1 (a major component of stress granules) and some other markers from a total of 12 fractions by immunoblotting. To isolate the RNPs from separated fraction we performed immunoprecipitation using TIA-1 antibody. To study the composition of protein and mRNA targets of RNPs, we will use mass spectrometry and RNA sequencing respectively.

Results: TIA-1 protein was enriched in the top fractions (2-5) however, some high molecular forms of TIA-1 most probably the oligomeric forms were observed in the bottom fractions (11-12). Furthermore, we identified the enrichment of other markers involved in Alzheimer's disease such as SFPQ, L1CAM, oligomeric tau, and phosphorylated tau.

Conclusions:

We successfully isolated the TIA-1-associated stress granules from human brain to unravel their composition and possible role in protein aggregation. Identifying the components of RNPs from the human brain can provide us with potentially novel targets for therapeutic interventions by dysregulating the pathological granules.

Analysis of mechanism for brain-region specific prion propagation in mice infected with prions

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Objective: PrP^{Sc} deposition, which is synonymous with prion propagation, is not evenly distributed in brains. Brain region-dependent PrP^{Sc} accumulation, this phenomenon implies a potential neuronal cell tropism of prion propagation. We conducted in vivo and ex vivo analyses to elucidate this mechanism.

Methods: PrP^{Sc} in mouse brains was detected with PrP^{Sc}-specific staining using monoclonal antibody (mAb) 132. The expression of *vGAT*, a marker for inhibitory GABAergic neurons, and *vGlut1* and *vGlut2*, markers for excitatory glutamatergic neurons, were analyzed using RNAscope in situ hybridization. Stereotactic injections of prions were performed into the thalamus and striatum, followed by immunohistochemical detection of PrP^{Sc}. To evaluate neuronal tropism, primary neuronal cultures of the cortex, thalamus, and striatum from 14-day ICR mouse embryos were prepared and inoculated with prions. Prion propagation in excitatory glutamatergic and inhibitory GABAergic neurons was assessed through multiple fluorescence staining using anti-PrP mAb 8D5 for PrP^{Sc} detection, and the "STAIN perfect – Immunostaining kit A" for glutamate and GABA detection.

Results: PrP^{Sc} accumulation was prominent in the cerebral cortex where *vGlut1*-expressing excitatory neurons existed and in the thalamus housing both *vGlut1* and *vGlut2*-expressing excitatory neurons. However, the striatum, rich in *vGat*-positive inhibitory neurons, exhibited moderate PrP^{Sc} accumulation. In stereotactic injection of prions to the thalamus and striatum, PrP^{Sc} stains that probably indicate prion propagation, were observed near the needle track and traumatic injury site in the thalamus but faint PrP^{Sc} staining was observed at the striatal 14 days post-inoculation. Immunofluorescence analysis of prion-infected primary neuronal cultures revealed that prions propagate more efficiently in GABA-negative excitatory neurons than GABA-positive inhibitory neurons.

Conclusions: Our findings suggest that prions propagatemore efficiency in glutamatergic excitatory neurons than in GABAergic inhibitory neurons. The

results shed light on the mechanisms of brain-region dependent propagation of prions.

Theme	(X)
Neuropathology of prion diseases	X
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

An expanded genetic code for native-state, live labelling of prions

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Aims: Fluorescent labelling methods for monitoring prions rely heavily on antibody-based approaches, which obviate the structural conversion of the cellular prion protein (PrP^C) into prions and often require fixation and permeabilisation of cells to work. The alternative, fluorescent fusion proteins of PrP^C, only allow compromised structural conversion and change the biochemical characteristics of converted material. As we delve deeper into the nanoscale of prion biology, artefacts produced by these labelling methods will become unavoidable. It is vital for further investigation of prion infection and conversion to develop a fluorescent labelling system which can label prions under live, native conditions in a way that minimally affects their biochemical characteristics.

Materials and Methods: Genetic code expansion (GCE) exploits bio-orthogonal translational components to site-specifically incorporate unnatural amino acids (UAA) into proteins by amber stop codon suppression. By using GCE to incorporate a UAA compatible with tetrazine-based click chemistry into PrP^C in a mouse neuroblastoma cell line and primary mouse cortico-hippocampal neurons, we have produced fluorescently labelled PrP^C (PrP_{amb}) with only a single amino acid modification.

Results: Considerable levels of PrP_{amb} are produced, and are capable of converting into disease-associated PrP (PrP^d). PrP_{amb} replicates typical biochemical characteristics of PrP^C and maintains normal distribution in cells, while being able to bind a fluorescent label under native conditions in live cells.

Conclusions: Using this system, PrP^d can be investigated at high-resolutions in its native state. We will use it to image vitrified prion-infected cells by cryogenic correlative light and electron microscopy (cryo-CLEM) to learn more about the micro-environments in which prions establish infection.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	(X)
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Therapeutic approaches for prion and prion-like diseases	

Insight into the molecular interaction between α-synuclein and prion protein

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Aims: The interaction between α-synuclein (aSyn) and prion protein (PrP) has been implicated in the pathogenesis of synucleinopathies. However, the nature of this interaction remains poorly understood. Here, we aimed at detailing the molecular mechanisms involved in the interaction between the two proteins.

Materials and Methods: We employed a combination of biophysical (NMR, SPR, fluorescence, DLS, calorimetry) and cellular assays to characterize the interaction between aSyn and PrP.

Results: We showed that PrP 23-231 interacts with aSyn in its monomeric, oligomeric, and fibril states. The binding was exothermic, and no interaction was observed with PrP 90-231. Our results demonstrate that the C-terminal region of aSyn interacts with PrP in an orientation-specific manner through electrostatic interactions, and suggest that tryptophan residues on PrP may also bind to the tyrosine residues of aSyn through π–π interactions. Specifically, interaction with aSyn fibrils induced PrP aggregation. We further demonstrate that PrP potentiates seeded aSyn aggregation kinetics, as evidenced by an increase in monomer incorporation rate in the real-time quaking-induced conversion assay. Additionally, we observed colocalization between PrP and phosphorylated aSyn, suggesting a possible role of PrP in aSyn phosphorylation.

Conclusions: Our findings provide valuable insights into the nature of the aSyn-PrP interaction and highlight potential therapeutic targets for synucleinopathies. In particular, targeting the C-terminal of aSyn or the N-terminal of PrP could be a promising strategy for preventing or reversing the effects of aSyn aggregation and spreading. Future studies could investigate the role of post-translational modifications

of aSyn and the conformational states of aSyn in the interaction with PrP, as well as the effect of aSyn aggregates formed via PrP-aSyn interaction on cellular toxicity and spreading.

Theme: Pathogenic mechanisms in prion and prion-like diseases

The detection and decontamination of chronic wasting disease prions during venison processing

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Aims: There is a growing concern that chronic wasting disease (CWD) prions in venison pose a risk to human health. CWD prions accumulate in infected deer tissues that commonly enter the human food chain through meat processing and consumption. The United States (US) Food and Drug Administration and US Department of Agriculture now formally consider CWD-positive venison unfit for human and animal consumption. Yet, the degree to which prion contamination occurs during routine venison processing is unknown. Here, we use environmental surface swab methods to: a) experimentally test meat processing equipment (i.e., stainless steel knives and polyethylene cutting boards) before and after processing CWD-positive venison and b) test the efficacy of five different disinfectant types (i.e., Dawn dish soap, Virkon-S, Briotech, 10% bleach, and 40% bleach) to determine prion decontamination efficacy.

Materials and Methods: We used a real-time quaking-induced conversion (RT-QuIC) assay to determine CWD infection status of venison and to detect CWD prions in the swabs. We collected three swabs per surface and ran eight technical replicates on RT-QuIC.

Results: CWD prions were detected on all cutting boards (n= 3; replicates= 8/8, 8/8, 8/8 and knives (n= 3; replicates= 8/8, 8/8, 8/8) used in processing CWD-positive venison, but not on those used for CWD-negative venison. After processing CWD-positive venison, allowing the surfaces to dry, and washing the cutting board with Dawn dish soap, we detected CWD prions on the cutting board surface (n= 3; replicates= 8/8, 8/8, 8/8) but not on the knife (n= 3, replicates = 0/8, 0/8, 0/8). Similar patterns were observed with Briotech (cutting board: n= 3; replicates= 7/8, 1/8, 0/8; knife: n= 3; replicates = 0/8, 0/8, 0/8). We did not detect CWD prions on the knives or cutting boards after disinfecting with Virkon-S, 10% bleach, and 40% bleach.

Conclusions: These preliminary results suggest that Dawn dish soap and Briotech do not reliably decontaminate CWD prions from these surfaces. Our data suggest that Virkon-S and various bleach concentrations are more effective in reducing prion contamination of meat processing surfaces; however, surface type may also influence the ability of prions to adsorb to surfaces, preventing complete decontamination. Our results will directly inform best practices to prevent the introduction of CWD prions into the human food chain during venison processing.

Acknowledgement: Funding was provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR), the Rapid Agriculture Response Fund (#95385/RR257), and the Michigan Department of Natural Resources.

Theme: Animal prion diseases

Itchy and clumsy sheep: clinical diagnosis of ovine transmissible spongiform encephalopathies

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Aims:
Scrapie in sheep is a notifiable transmissible spongiform encephalopathy (TSE). For countries that do not implement active surveillance for scrapie, monitoring of scrapie prevalence is purely based on reporting of clinical suspects. This requires a good knowledge of the clinical presentation, which is often difficult because of the rarity of the disease.
A protocol had been developed at APHA Weybridge to aid in the clinical diagnosis of various TSEs, including atypical scrapie, concentrating on specific clinical signs. This study aimed to determine the usefulness of this protocol by selecting clinical markers of importance for a clinical diagnosis of scrapie in sheep.
Material and Methods:
A total of 710 sheep were included in the study, of which 274 (38.6%) were naturally or experimentally infected with a TSE agent: classical scrapie (173, 63.1%), atypical scrapie (53, 19.3%) or Bovine Spongiform Encephalopathy (48, 17.5%) and had a brain-positive TSE test result. Assessed signs by a single operator were behaviour, blindfolding response, body condition, coordination, menace response, posture, scratch test response, tremor as well as wool loss and skin lesions. A general classification and regression tree model was used to determine the clinical markers of importance, omitting those signs of lesser importance without affecting diagnostic sensitivity or specificity.
Results:
The model revealed that the main clinical markers to predict a scrapie case were a positive scratch test and the presence of incoordination in case of a negative scratch test. This test protocol was 82.8% sensitive and 93.3% specific with a positive and negative predictive value of 88.7% and 89.6% respectively.
Conclusions:
Whilst the protocol relying on only two clinical signs is simple to implement with an adequate diagnostic accuracy for a clinical test, interpretation of clinical signs can be subjective, and it does not necessarily replace a neurological examination to determine location of lesion and possible alternative diagnoses.

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	**X**
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Unraveling the Influence of Prion Protein on Brain Mitochondrial Calcium Dynamics

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Introduction/Aims: Cellular prion protein (PrP^C) is mainly present in the plasma membrane of neural cells and is known to participate in several intracellular signaling pathways. In 2016, the presence of PrP^C in the inner membrane of brain mitochondria was demonstrated, which still needs to be explored in the scientific literature. The present study seeks to investigate the role of PrP^C in the physiology of brain mitochondria, focusing on mitochondrial calcium dynamics.

Materials and Methods: To assess the role of PrP^C on mitochondrial physiology, we performed experiments using brain mitochondria isolated from C57BL6 Wild-type (WT) versus PrP^C knock-out (PrP-KO) mice. To assess oxygen flux, we used high-resolution respirometry in Oroboros O2k. To investigate mitochondrial calcium uptake, we evaluated the fluorescence of the Calcium Green 5N calcium-binding probe in a Cary Eclipse fluorometer. Using Western blot, we also measured the expression of mitochondrial calcium uniporter (MCU) and mitochondrial hexokinase (mt-HK) and evaluated the OxPhos-coupled activity of mt-HK spectrophotometrically.

Results: No difference was observed between the real-time oxygen flow of WT and PrP-KO mitochondria. Nevertheless, our experiments on calcium uptake revealed a shift towards calcium retention in PrP-KO brain mitochondria compared to WT, culminating in a significantly higher concentration of calcium in PrP-KO mitochondria. This modulation does not involve alteration in the expression of MCU nor mt-HK, an essential modulator of calcium uptake by brain mitochondria. The enzymatic activity of mt-HK is also similar between WT and PrP-KO mitochondria. Liver mitochondria from PrP-KO mice also presented a higher rate of calcium uptake when compared to WT.

Conclusion: Our findings suggest that PrP^C plays a role in regulating mitochondrial calcium dynamics. However, further experiments are required to fully comprehend the molecular mechanism of how PrP affects mitochondrial function in physiological processes.

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Acknowledgment: Funding agencies FAPERJ, CAPES, and CNPq

Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	X
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Pharmacokinetics and mechanisms of action of Anti-PrP antibody AZ59

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Aims: Antibodies are a safe and versatile tool against human diseases, and have recently seen advances for brain disease. In the context of prion disease, key questions remain around pharmacology and mechanism of anti-PrP antibodies *in vivo*. We aim to elucidate these parameters for one such antibody, AZ59.
Material and Methods: We injected WT and PrP KO mice intravenously with AZ59. We then evaluated AZ59 pharmacokinetics in presence and absence of the target. In addition, we quantified PrP in the brain and in the periphery (colon) to monitor target engagement.
Results: AZ59 exhibited non-linear pharmacokinetics exclusively in WT mice, where a 1 mg/kg dose achieved only 0.1% the drug exposure of a 100 mg/kg dose. In contrast, in PrP KO mice, AZ59 pharmacokinetics were linear, with a 100-fold difference between 1 mg/kg and 100 mg/kg groups, and overall drug exposure was higher than in WT mice. Together, these data suggest that the AZ59-PrP complex undergoes target-mediated drug disposition (TMDD), a phenomenon whereby the antibody's binding of its target causes its degradation. Surprisingly, PrP concentration increased in the colon after 100 mg/kg and in the brain after 780 mg/kg AZ59, suggesting that binding may have led to relocalization or sequestration rather than immediate degradation. This hypothesis is supported by observed accumulation of AZ59 in the colon.
Conclusion: The different doses required to alter PrP concentration in colon and brain may reflect two factors: 1) differing levels of PrP expression in each tissue, with measurable increase occurring only when a substantial proportion of PrP has been saturated by antibody, and 2) the challenge of delivering antibodies across the blood brain barrier. Regarding the mechanism, we hypothesize that AZ59 is sequestering PrP at the cell surface delaying its degradation. The relevance of this mechanism to potential anti-prion activity remains to be determined.

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Theme: therapeutic approaches for prion and prion-like diseases

Genome wide CRISPR activation screen to identify genetic modifiers of prion replication

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Aims: In this study, we performed a genome-wide CRISPR/Cas9 activation screen in human prion-infected cells to identify genes regulating prion replication.

Materials and Methods: Chronically human prion-infected cells expressing the transactivator dCas9-VP64 were infected with our in-house generated CRISPR library and prion levels were quantified with a PrPSc-specific antibody at two different timepoints after infection. The cells with high or low fluorescence corresponding to high or low levels of PrPSc were sorted and sequenced to identify the gRNAs of the genes that were enriched or dropped out in these populations.

Results: We have generated a list of potential genetic candidates that play a role in the process of prion replication and were not previously linked to prion disease.

Conclusions: Next steps include technical validation of the genes and further evaluation in a variety of model systems (different cell lines modeling prion disease, mice organotypic brain slices) to find the strongest genetic candidates with a universal effect on prion replication.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	X
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Engineering of a single chained fluobody into a flashbody format for optimized detection and labeling of native PrP^{Sc}

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Aims:

Fluorescent probes associated with single-chain antibodies (scFv) offer a unique method to detect prions from various prion isolates. Fluobody, a scFv based on the conformation-dependant PrP^{Sc}-specific monoclonal antibody YEG Sc-G1 (G1) fused with eGFP, revealed widespread surface labelling of PrP^{Sc} on RML-infected cells and minimal fluorescence in uninfected cells. However, generating fluorescence only upon antigen binding is more desirable. The goal of this study was to engineer, a PrP^{Sc}-specific "flashbody" probe, a tool for enhanced, antigen-specific fluorescent detection of native PrP^{Sc} to offer insights into prion replication.

Methods:
The fluorescent "fluobody" and "flashbody" antibody constructs were engineered from the previously characterized monoclonal antibody G1, which detects only native PrP^{Sc}. eGFP was fused to the C-terminus of the G1 scFv or between the variable light and heavy chains of the scFv, to create the fluobody and flashbody constructs, respectively. The probes were expressed in *E. coli* and purified via metal-ion affinity chromatography, before being assessed using immunocytochemistry.

Results:
Preliminary results from immunocytochemistry revealed enhanced surface labelling of PrP^{Sc} aggregates in RML-infected organotypic brain slices and CAD5 cells. Uninfected controls had significantly reduced, unspecific labelling. Compared to the fluobody, the flashbody showed lower levels of background fluorescence and a greater variability in punctae size and intensity, suggestive of an antigen-antibody dependant fluorescence. The variable foci are suggestive of different quaternary structures.

Conclusions:
Conjugating a scFv with eGFP is a powerful strategy for imaging protein aggregates in living cells or lightly fixed tissues without the need for secondary detection systems. The PrP^{Sc}-specific flashbody promises to be a highly beneficial tool versus the previously presented fluobody and regular, linear epitope antibodies due to its enhanced specificity, selectivity, and affinity for native PrP^{Sc}. These probes may act as diagnostic tools and show promise in the characterization the protein structures adopted during prion propagation.

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Theme (X)
Neuropathology of prion diseases
Functional protein aggregation in yeast and mammalian systems
Protein structure, function, conversion, and dysfunction (X)
Spreading of pathology in prion-like disorders
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Animal prion diseases
Biomarkers for prion and other neurodegenerative diseases
Therapeutic approaches for prion and prion-like diseases

Expression of Rps24-PKE is induced by activated microglia during acquired and genetic prion diseases

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Aims: To find novel biomarkers or therapeutic targets, we recently performed cell type-specific translatome studies in four mouse models of pre-symptomatic neurodegenerative disease. Changes in ribosome protein expression led us to hypothesize that specialized ribosomes are involved. The mRNA encoding Rps24 (small subunit ribosome protein 24) normally undergoes tissue and cell type-specific alternative splicing, resulting in three C-terminal protein ends. Antibodies against the -PKE isoform were predicted to be useful in detecting neurodegeneration. We also sought to characterize Rps24-PKE expression in blood as it might be useful as a blood-based biomarker.

Materials and Methods: Antibodies that detect Rps24-PKE were generated in mice and rabbits. Antibodies were validated by western blot analysis of lysates from cells genetically engineered to express each of the main Rps24 isoforms. Once validated, they were used to examine brain histological sections from mice and humans with neurodegenerative diseases. In preliminary experiments to determine if Rps24-PKE can be used as a biomarker, white blood cells from healthy and leukemic patients were analyzed by digital droplet PCR and immunocytochemistry.

Results: Our antibodies bound specifically to the brains of humans and mice with neurodegeneration. The reactivity was most prominent in activated microglia and macrophages. In cytological spreads of white blood cells, the antibodies demonstrated a wide distribution of Rps24-PKE expression levels from strong in some cells to no expression in others. Expression of the mRNA isoform encoding Rps24-PKE tracks with progression of chronic lymphocytic leukemia.

Conclusions: These novel antibodies demonstrate that Rps24-PKE is expressed in many neurodegenerative diseases, most prominently in microglia and macrophages. It appears to represent a novel marker specific to these cell types when activated. Initial studies also show that expression of Rps24-PKE in white blood cells changes in response to disease and that studies into its potential as a biomarker for neurodegenerative diseases are warranted.

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Theme	(X)
Neuropathology of prion diseases	x
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	

Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	X
Therapeutic approaches for prion and prion-like diseases	

Title: Transmission of atypical BSE: a possible origin of Classical BSE in cattle

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Background: Bovine spongiform encephalopathy (BSE) is a fatal neurodegenerative disease of cattle and is categorized into classical and atypical forms. Classical BSE (C-BSE) is linked to the consumption of BSE contaminated feed whereas atypical BSE is considered to be spontaneous in origin. The potential for oral transmission of atypical BSE is yet to be clearly defined.

Aims: To assess the oral transmissibility of atypical BSE (H and L type) in cattle. Should transmission be successful, determine the biochemical characteristics and distribution of PrP^{Sc} in the challenge cattle.

Material and Methods: For oral transmission, calves were fed with 100 g of either H (n=3) or L BSE (n=3) positive brain material. Two years post challenge, 1 calf from each of the H and L BSE challenge groups exhibited behavioural signs and were euthanized. Various brain regions of both animals were tested by traditional and novel prion detection methods with inconclusive results. To detect infectivity, brain homogenates from these oral challenge animals (P1) were injected intra-cranially (IC) into steer calves. Upon clinical signs of BSE, 3/4 of IC challenged steer calves were euthanized and tested for PrP^{Sc} with ELISA, immunohistochemistry and immunoblot.

Results: After 6 years of incubation, 3/4 animals (2/2 steers IC challenged with brain from P1 L-BSE oral challenge and 1/2 steer IC challenged with brain from P1 H-BSE oral challenge) developed clinical disease. Analysis of these animals revealed high levels of PrP^{Sc} in their brains, having biochemical properties similar to that of PrP^{Sc} in C-BSE.

Conclusion: These results demonstrate the oral transmission potential of atypical BSE in cattle. Surprisingly, regardless of which atypical type of BSE was used for P1 oral challenge, PrP^{Sc} in the P2 animals acquired biochemical characteristics similar to that of PrP^{Sc} in C-BSE, suggesting atypical BSE as a possible origin of C-BSE in UK.

Presentation Type: Oral Presentation

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Theme: Animal prion diseases

Large-Scale Validation of Skin Prion Seed-Amplification Assay for Diagnosis of Creutzfeldt-Jakob Disease

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Abstract

Aims

Our previous study revealed that PrP^{Sc}-seeding activity (PrP^{Sc}-SA), a peculiar feature of misfolded PrP^{Sc}, can be detected in skin tissues of sCJD patients with high sensitivity and specificity by an ultrasensitive PrP^{Sc} seed-amplification assay (PrP^{Sc}-SAA) known as real-time quaking-induced conversion (RT-QuIC).

Materials and Methods

875 skin samples were retrospectively and prospectively collected at autopsy from three body areas of 335 individual cases with different neuropathologically confirmed subtypes of sCJD and non-sCJD controls. These samples were analyzed for skin PrP^{Sc}-SA by RT-QuIC in two independent laboratories. The results were compared with demographic information, clinical manifestations, cerebrospinal fluid (CSF) PrP^{Sc}-SA, and other laboratory tests including CSF 14-3-3 and total tau in deceased patients with and without sCJD.

Results

The 335 cases included in this study were composed of retrospective (n=121) and prospective (n=214) cohorts. The retrospective cohort was tested by RT-QuIC unblinded in one lab and validated blindly in an independent lab. RT-QuIC assays of the retrospective cohort in the two laboratories gave the same 84.5% sensitivity and 100% specificity. The prospective cohort showed sensitivity of 83.2% and specificity of 97.3% by RT-QuIC. Analysis of CSF available

from 212 cases gave 89.7% sensitivity and 94.4% specificity. The sensitivity of skin RT-QuIC was subtype-dependent, being highest in sCJDVV1-2 subtype, followed by VV2, MV1-2, MV1, MV2, MM1, MM1-2, MM2, and VV1. Of the dermatomes, the skin area next to the ear gave highest sensitivity, followed by lower back and apex of the head. Although no difference in brain PrP^{Sc}-SA was detected between the cases with false negative and true positive skin RT-QuIC results, the disease duration was significantly longer with the false negatives [12.0 ± 13.3 (months, SD) vs 6.5 ± 6.4, $p < 0.001$].

Conclusions

Our study validates skin PrP^{Sc}-SA as a biomarker for the detection of prion diseases.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

Theme	Selection
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
Protein structure, function, conversion, and dysfunction	
Spreading of pathology in prion-like disorders	
Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	X
Therapeutic approaches for prion and prion-like disease	

Prophylactic and Therapeutic Efficacy of a Cellulose Ether Compound TC-5RW on Acquired and Spontaneous Prion Diseases in Humanized Transgenic Mice

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Abstract

Aims

The long-term aim of our study is to develop effective prevention and treatment of human prion diseases. The cellulose ethers (CEs) including TC-5RW have been reported to have prophylactic and therapeutic effects against animal prions in rodents. Our previous study has revealed that TC-5RW is also able to inhibit the seeding activity of the infectious human prion protein (PrP^{Sc}) and to directly decrease the level of proteinase K (PK)-resistant PrP^{Sc} upon incubation of TC-5RW with brain homogenates of patients with different sporadic and genetic CJD *in vitro*. This study aspired to test our hypothesis that TC-5RW has prophylactic and therapeutic effects on human prion diseases.

Materials and Methods

To determine the effect of the TC-5RW compound on the acquired and spontaneous forms of prion diseases, we examined the compound with two lines of animal models: 1) Tg40h expressing wild-type human PrP carrying methionine (M) at residue 129 to mimic the acquired human prion disease upon intracerebral inoculation with sporadic CJD (sCJD) brain homogenate and 2) TgMHu2ME199K mice expressing chimeric mouse-human PrP with E199K mutation to mimic the spontaneous genetic human prion disease CJD^{E200K}. To explore the prophylactic effect, Tg40h mice (prophylactic group, PG) were subjected to a single subcutaneous administration of TC-5RW at 4 g/kg body weight (BW) 2 weeks before intracerebral inoculation with brain homogenate from a cadaver with sCJDMM1. To investigate the therapeutic effect, another group of mice (therapeutic group, TG) was subjected to a single subcutaneous administration of the same amounts of TC-5RW 5 months after intracerebral inoculation with brain homogenate from

sCJDMM1. For TgMHu2ME199K, the mice were given a single subcutaneous administration of TC-5RW at 4 g/kg BW at 2 months of age (PG) for evaluating prophylactic effect while the mice were subjected to a single subcutaneous administration of the same amounts of compound at 7 months of age (TG). Both lines of animals with no TC-5RW treatment were used as controls. Each group contained 14-16 mice, and they were monitored 3 days a week. At the end of the experiments, the brain tissues were examined by histology and western blotting with anti-PrP antibodies.

Results

The Tg40h mice from the prophylactic group showed an increased survival time compared to sham-treated controls [214.9 days post-inoculation (dpi) ± 5.2 vs 206.5 ± 10.1 (dpi), $p < 0.01$). In contrast, no significant difference was observed between the mice treated with TC-5RW after prion exposure and sham-treated mice (208.3 ± 10.7 vs 206.5 ± 10.1 dpi, $p = 0.89$). The amount of brain PK-resistant PrP^{Sc} was significantly decreased in PG than in control mice [arbitrary unit 22848 ± 10183 vs 56011 ± 4159 au, $p < 0.001$]. It was also decreased in the TG mice but it did not reach the degree of statistically significant difference compared to the controls (au: 38852 ± 13977 vs 56011 ± 4159, $p = 0.057 > 0.05$). For the TgMHu2ME199K mice, they were monitored for disease severity and progression according to a scale of clinical signs developed previously (Friedman-Levi et al., 2011), in which the mice with the lowest score of 0 had no clinical signs while the mice with the highest score 4 exhibited full paralysis in both limbs. The score of mice with early TC-5RW treatment (PG) was significantly lower than mice without the compound treatment at day 169 [2.07 ± 0.47 vs 3.0 ± 0.77 au, $p = 0.002 < 0.005$]. The score of mice with late TC-5RW treatment (TG) was also lower compared to untreated mice at day 401 (3.12 ± 0.5 vs 3.64 ± 0.6 au, $p = 0.026 < 0.05$). The amounts of PK-resistant PrP^{Sc} in the brain of mice

treated early with TC-5RW at month 3 was significantly reduced compared to the untreated mice (1138.7 ± 588.8 vs 2814.1 ± 659.9 a.u., $p = 0.00017 < 0.0005$). But the amounts of PK-resistant PrP^{Sc} in the brain of mice treated with TC-5RW at month 7 showed no significant difference compared to control mice (2368.2 ± 556.8 vs 2814.1 ± 659.9 au, $p = 0.179 > 0.05$).

Conclusions

Our study suggests that TC-5RW is of the potential to serve as a prophylactic compound to prevent human prion disease; however, the therapeutic effect of the compound on the two lines of animal models is less effective.

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Theme	Selection
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
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Pathogenic mechanisms in prion and prion-like diseases	
Animal prion diseases	
Biomarkers for prion and other neurodegenerative diseases	

Therapeutic approaches for prion and prion-like disease	X

Copper ions modulate prion protein phase transitions

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Prion diseases are characterized by prion protein (PrP) transmissible aggregation and toxicity in the brain. The physiological function of PrP seems related to sequestering of redox-active Cu^{2+}, and Cu^{2+} dyshomeostasis is observed in prion disease brain. It is unclear whether Cu^{2+} contributes to PrP aggregation, recently shown to be mediated by PrP condensation. We investigated the role of Cu^{2+} and oxidation in PrP condensation and aggregation using multiple biophysical and biochemical methods. We find that Cu^{2+} promotes PrP condensation at the cell surface and in vitro through co-partitioning. Molecularly, Cu^{2+} inhibited PrP β-structure and hydrophobic residues exposure. Oxidation, induced by H_2O_2, triggered liquid-to-solid transition of PrP:Cu^{2+} condensates and promoted amyloid-like PrP aggregation. In cells, overexpression of PrP^C initially protected against Cu^{2+} cytotoxicity but led to PrP^C aggregation upon extended copper exposure. Our data suggest that PrP condensates function as a buffer for copper that prevent copper toxicity but can transition into PrP aggregation at prolonged oxidative stress.

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Theme	(X)
Neuropathology of prion diseases	
Functional protein aggregation in yeast and mammalian systems	
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Relationship between hyperosmotic stress, liquid-liquid phase separation and prion formation

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Aims: Proteins with intrinsically disordered regions may both undergo liquid-liquid phase separation (LLPS) and form solid fibrous aggregates (amyloids). LLPS is involved in the spatiotemporal control of crucial cellular functions, while amyloids are associated with a variety of diseases and can manifest themselves as transmissible elements (prions). Even though some aberrant phase transitions have been linked to amyloid formation, the overall relationship between LLPS and amyloid formation *in vivo* is poorly understood. The goal of this work is to decipher this relationship using the yeast protein, Sup35, as a model.

Materials and Methods: Fluorophore-tagged protein constructs were expressed in *Saccharomyces cerevisiae* cells, and the formation of liquid condensates and amyloid fibrillar assemblies was monitored at various expression levels using both fluorescence microscopy and biochemical/biophysical analysis. Generation of heritable prions was detected by phenotypic assays.

Results: Hyperosmotic stress and/or protein overproduction promote LLPS of Sup35-based constructs in cells lacking pre-existing prions. Formation of Sup35 prions is also increased by hyperosmotic stress. The prion domain (Sup35N) is both necessary and sufficient for LLPS, while the middle domain (Sup35M) antagonizes LLPS. Sup35's ability to undergo LLPS in response to hyperosmotic stress is conserved among various yeast species. Liquid condensates produced by Sup35N/NM of the distantly related yeast species, *Ogataea methanolica*, in osmotically stressed *S. cerevisiae* cells can directly convert to fibrillary amyloids. Some mammalian amyloidogenic proteins, such as 2N4R isoform of tau, also undergo LLPS in yeast cells.

Conclusions: Our data uncover the intimate connection between stress-induced LLPS and the formation of transmissible amyloid-based prions.

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Theme	(X)
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Biomarkers for prion and other neurodegenerative diseases	
Therapeutic approaches for prion and prion-like diseases	

Molecular phenotype shift after passage of low-type bovine spongiform encephalopathy (L-BSE).

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Aims: The purpose of this study is to compare the molecular phenotypes of L-BSE in wild type cattle and cattle with the E211K polymorphism to samples of other cattle TSEs, such as classical BSE (C-BSE), high-type BSE (H-BSE), and transmissible mink encephalopathy (TME).

Materials and Methods: Two wild type cattle (EE211 *PRNP*) and one steer with the E211K polymorphism (EK211) were intracranially inoculated with 1 mL of brain homogenate that originated from a 2005 French L-BSE case. Multiple assays were used to compare and differentiate tissues, including enzyme immunoassay, western blot (Sha31, 12B2, SAF84), stability (Sha31), and immunohistochemistry (F99/97).

Results: Approximately 16.6 months post-inoculation, Steer 6 (EK211 L-BSE) developed neurologic signs, including agitation, difficulty eating accompanied by weight loss, head tremor, ataxia, and fasciculations in the forelimbs, and was necropsied. Enzyme immunoassays demonstrated misfolded prion protein in the brainstem (4.0 O.D) but not in peripheral tissues, such as the retropharyngeal lymph node and palatine tonsil. When compared by western blot, the molecular phenotype of the brainstem of Steer 6 (EK211 L-BSE) is higher than that of wildtype cattle inoculated with L-BSE, requiring careful differentiation from C-BSE. Ongoing mouse studies in bovinized mice (K211 and TgBov) will provide data to compare to all other BSE strains available, including L-BSE, C-BSE, H-BSE, E211K H-BSE, and TME.

Conclusions: Further study of L-BSE in EK211 cattle with a higher molecular phenotype in the brainstem may give more insight into the origin of C-BSE.

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Theme: Animal prion diseases

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