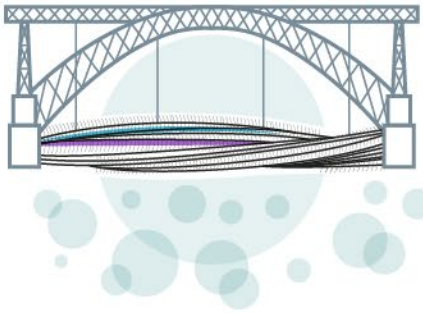

13th Iberian Congress on Prions

ICBAS, Oporto

Oporto, May 22 - 23, 2025

Abstract Book

13th Iberian Congress on Prions 2025

22nd-23rd May 2025

Instituto de Ciências Biomédicas Abel Salazar (ICBAS) da Universidade do Porto (UP)" organizing with the "Instituto Nacional de Investigação Agrária e Veterinária (INIAV)" and the "Sociedade Portuguesa de Ciências Veterinárias (SPCV)" welcome all in the Anfiteatro 5.

Organization

Sponsorship

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- E. Vidal | IRTA-Cresa, UAB, Barcelona
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- I. Zerr | University of Gottingen
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13th Iberian Congress on Prions
ICBAS, Oporto
22nd and 23rd of May 2025

PROGRAM

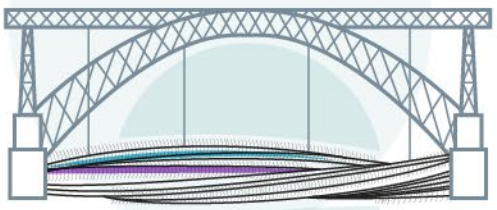
THURSDAY, 22nd MAY

13:00-14:00	Registration
14:00	Welcome
	PRION STRUCTURE AND BIOLOGY
14:30-15:30	**Chairperson**: Jesus Requena
	Invited Speaker: Dr Hasier Eraña (Center for Cooperative Research in Biosciences (CIC BioGUNE), Basque Research and Technology Alliance (BRTA), Derio, Spain, Centro de Investigación Biomédica en Red de Enfermedades infecciosas (CIBERINFEC), Carlos III National Health Institute, Madrid, Spain; ATLAS Molecular Pharma S. L, Derio, Spain) *Dissecting natural resistance to prion disease: from phylogenetic insight to transgenic models*
15:30-16:30	**Chairpersons**: Nuno Anjo and Carlos Díaz
	Oral Communications: 1. Emiliano Biasini (CIBIO, University of Trento) **"Preclinical Development of Drug-Like Compounds Halting Prion Neurotoxicity"** 2. Francesca Peccati (CIC bioGUNE, Ikerbasque) **"Structural basis for glycoform ratio specificity among prion strains"** 3. Raul C. Vazquez (CIMUS, University of Santiago de Compostela) **"Structural Dynamics and Kinetic Interplay in Prion Assembly"** 4. Juan-Carlos Espinosa (CISA-INIA-CSIC) **"Spontaneous neurodegenerative disease in a transgenic mouse model expressing H95Y PrP-amino acid change"**
16:30-17:00	Coffee Break and Poster Discussion
17:00-18:30	**Chairpersons**: Enric Vidal and Leonor Orge
	Oral Communications: 5. Sanaz Sabzehei (CIMUS, University of Santiago de Compostela) **"Unfold to refold: exploring the early events of PrP^{C} to PrP^{Sc} conversion with solution NMR and Molecular Dynamics"** 6. Camille Zany (Université Paris Saclay, INRAE) **"Self-Organized Spatial Patterns of Prion Replication in Organotypic Cerebellar Slices Infected with the 127S Strain"**
	PRION DISEASES IN ANIMALS 7. Nerea L. Martínez (Centro de Encefalopatías y Enfermedades Transmisibles Emergentes, Universidad de Zaragoza) **"Gene therapy for prion diseases using an adeno-associated viral vector"** 8. Carlos M. Díaz-Domínguez (CIC BioGUNE, Prion Research Center, Colorado State University) **" Synthetic Cervid Prions in Gt Mice as a Model for Novel CWD Strain Emergence and Evolution"** 9. Joseph P. DeFranco (Prion Research Center. Colorado State University) **"Different transmissible properties of CWD prions from North America and Northern Europe in cervidized gene-targeted mice"** 10. Diana C. Lowe (Prion Research Center, Colorado State University) **"Remarkable diversity of emergent CWD strains in Swedish moose"**
18:30-19:00	**Supporting Associations of prion diseases patients**

FRIDAY, 23rd May

PRION DISEASES IN ANIMALS	
9:00-10:00	**Chairperson**: Sylvie Benestad **Invited Speaker: Dr Fiona Houston** (University of Edinburgh) *Prions in Blood: Lessons from a Large Animal Model*
10:00-10:30	Coffee Break and Poster Discussion
10:30-12:30	**Chairpersons**: Vera Silva and Juan Maria Torres **Oral Communications:** 11. Hermann M. Schatzl (Calgary Prion Research Unit, University of Calgary) **"Zoonotic potential of chronic wasting disease prions demonstrated by passage in non-human primates and rodents"** 12. Lars A. Folkman (Norwegian University of Life Sciences) **"Transmission of reindeer chronic wasting disease to sheep"** 13. Enric Vidal (IRTA, CReSA, Universitat Autònoma de Barcelona) **"Generation of bona fide Mongolian gerbil (*Meriones unguiculatus*) prions in vitro"** 14. Stefania Thorgeirsdottir (Institute for Experimental Pathology at Keldur, University of Iceland) **"Case-control studies in scrapie flocks and recent findings on protective prion protein genotypes in Iceland"** 15. Timm Konold (Animal & Plant Health Agency) **"Eradication of scrapie in a goat herd by breeding for resistance"** 16. Lucien J.M. van Keulen (Wageningen Bioveterinary Research) **"Co-presence of classical scrapie 22A but not classical BSE in Dutch sheep with atypical scrapie"** 17. Sara Canoyra (CISA-INIA-CSIC) **"Prion evolution of Nor98/Atypical Scrapie in a homologous ovine PrP^C Context"** 18. Giuseppe Ru (Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta) **"Large-scale association study on the combined effect of genetics and age on atypical scrapie risk in the Portuguese sheep population"**
12:30-14:00	Lunch & Posters & Group photo
PRION DISEASES AND PRION-LIKE DISEASES IN HUMANS	
14:00-15:00	**Chairperson**: Inga Zerr **Invited Speaker: Dr Joel Watts** (Tanz Centre for Research in Neurodegenerative Diseases and University of Toronto) *α-Synuclein strains as drivers of disease heterogeneity*
15:00-16:15	**Chairpersons:** Jorge Moreno and Tomas Barrio **Oral Communications:** 19. Irantzu Pallarès (Universitat Autònoma de Barcelona) **"HeliCure targets α-synuclein oligomers and restores motor function in a preclinical mouse model of Parkinson's disease"** 20. Susana Silva Correia (National Reference Center for TSE, University Medical Center) **"Advancing prion diagnostics: Novel RT-QuIC substrate enables detection in tear fluid and enhances cerebrospinal fluid sensitivity"** 21. Inga Zerr (University of Göttingen) **"PrionPro: An International Quality Control Program for Prion Disease Biomarkers**

	22. **Rodrigo Morales** (University of Texas Health Science Center at Houston) **"Host-dependent variability in amyloid-beta propagation and deposition"** **23.** **Roberto Chiesa** (Department of Neuroscience, Istituto di Ricerche Farmacologiche Mario Negri) **"Tracking disease progression in fatal familial insomnia: Longitudinal plasma biomarker analysis"**
16:15-16:45	Coffee Break and Poster Discussion
16:45-18:15	**Chairperson: Joaquin Castilla**
	Invited Speaker: Dr Nina Oberbeck (GATE Bioscience) *Oral Small Molecules for Reducing Prion Protein Levels: A New Therapeutic Strategy for Prion Disease* **Next Meeting announcement and closing of the meeting**
19:30	Social event and Congress Dinner



Invited Speakers

Dr. Hasier Eraña

(Center for Cooperative Research in Biosciences (CIC BioGUNE), Basque Research and Technology Alliance (BRTA), Derio, Spain, Centro de Investigación Biomédica en Red de Enfermedades infecciosas (CIBERINFEC), Carlos III National Health Institute, Madrid, Spain; ATLAS Molecular Pharma S. L, Derio, Spain)

Dr. Hasier Eraña earned a Degree in Biotechnology from the Autonomous University of Barcelona in 2010. In 2011, he received a Master's degree in Microbiology from the same university, working in Dr. Ester Julián's laboratory to evaluate the use of mycobacteria in bladder cancer treatment. This role provided training in microbiology, cell culture models, immunoassays (ELISA), and flow cytometry.

Following his Master's, Hasier pursued a PhD in Molecular Biology and Biomedicine at the University of the Basque Country (2015), conducted at CIC bioGUNE under Dr. Joaquín Castilla's supervision and supported by a Basque Government grant (BFI-2010-7). His doctoral research focused on the molecular determinants of interspecies prion transmission, as described in his thesis, "Study of the Molecular Determinants that Modulate the Resistance of Rabbits to Prion Infection Using an In Vitro Propagation Model." This work advanced his expertise in *in vitro* prion propagation, ultrasensitive detection by PMCA, recombinant protein production, and biochemical analysis.

During this period, he also enhanced his skills on prion disease research through a short-term stay at Dr. Christina Sigurdson's laboratory at the University of California, San Diego, where he received training in neuronal cell culture models of prion disease and pathological analyses. Accredited for research with experimental animal models during his PhD, after completing it, Dr. Eraña continued as a postdoctoral researcher in Dr. Castilla's laboratory, focusing on interspecies prion transmission, prion strain characterization, and the development of *in vitro* prion propagation systems such as PMSA and new transgenic mouse models of prion disease. He currently serves as Project Manager at ATLAS Molecular Pharma S.L., a biotech company dedicated to developing therapies for rare diseases, including prion disorders, combining his work in Atlas with his research activities at the Prion Research Laboratory from CIC bioGUNE led by Dr. Castilla, and teaching at Deusto University.

Dissecting natural resistance to prion disease: from phylogenetic insight to transgenic models

Hasier Eraña[1,2,3], Jorge M. Charco[1,2,3], Cristina Sampedro-Torres-Quevedo[1,2], Francesca Peccati[1], Eva Fernández-Muñoz[1], Maitena San-Juan-Ansoleaga[1], Nuno Gonçalves-Anjo[1], Enric Vidal[4,5], Ganeko Bernardo-Seisdedos[6], Carlos M. Díaz-Domínguez[1], Josu Galarza-Ahumada[1], Africa Manero-Azua[7], Diego Polanco-Alonso[7], Samanta Giler[5,6], Diego Espada-Musitu[7], Manuel A. Sánchez-Martín[8], Mariano Domingo[5,9], Marivi Geijo[10], Guiomar Perez de Naclares[7], Gonzalo Jiménez-Osés[1], Jesus R.Requena[11], and Joaquín Castilla[1,2,12]

[1] Center for Cooperative Research in Biosciences (CIC BioGUNE), Basque Research and Technology Alliance (BRTA), Derio, Spain; [2] Centro de Investigación Biomédica en Red de Enfermedades infecciosas (CIBERINFEC), Carlos III National Health Institute, Madrid, Spain.
[3] ATLAS Molecular Pharma S. L., Derio, Spain; [4] IRTA. Programa de Sanitat Animal. Centre de Recerca en Sanitat Animal (CReSA). Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, Catalonia. Spain; [5] Unitat mixta d'Investigació IRTA-UAB en Sanitat Animal. Centre de Recerca en Sanitat Animal (CReSA). Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, Catalonia. Spain; [6] University of Deusto. Area of Health Sciences, Department of Medicine, Bilbao, Spain; [7] Molecular (Epi)Genetics Laboratory, Bioaraba Health Research Institute, Araba University Hospital, Vitoria-Gasteiz, Spain; [8] Transgenic Facility, Department of Medicine, University of Salamanca, Salamanca, Spain; Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain; [9] Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra, Spain; [10] Animal Health Department, NEIKER-Basque Institute for Agricultural Research and Development. Basque Research and Technology Alliance (BRTA), Derio, Spain; [11] CIMUS Biomedical Research Institute & Department of Medical Sciences, University of Santiago de Compostela-IDIS, Santiago de Compostela, Spain; [12] Ikerbasque, Basque Foundation for Science, Bilbao, Spain.

Recent work from our laboratory has uncovered a naturally evolved mechanism of prion disease resistance in cetaceans—whales, dolphins, and porpoises—that offers exciting insights into the structural constraints of prion propagation and potential new avenues for therapy. In our previous study, we analyzed the misfolding potential of over 900 mammalian PrP sequences and thanks to their grouping in phylogenetic clades, we found that cetacean PrPs are uniquely and invariably resistant to spontaneous misfolding. We have now elucidated that a single threonine insertion at position 194 within the cetacean PrP is both necessary and sufficient to prevent misfolding and propagation of prions.

By introducing this insertion into diverse mammalian PrP sequences, we observed a complete abolition of spontaneous misfolding *in vitro*. Our investigations further revealed that this protective effect is amino acid-specific, with certain residues conferring similar protection while others had minimal impact, and that the native tetrathreonine motif is dispensable for misfolding, underscoring the critical role of spatial positioning. To validate our findings *in vivo*, we developed transgenic mouse models carrying one or two threonine insertions, which demonstrated significant, strain- and dose-dependent protection against prion infection. Notably, while a single insertion delayed disease onset considerably, the double insertion not only extended incubation periods dramatically but also conferred complete resistance against certain prion strains. Moreover, NMR studies confirmed that these modifications preserve the native PrP structure, indicating a selective interference with pathological conversion.

In this talk, we will discuss the mechanistic insights from these findings, their implications for understanding prion propagation and evolution, and how they might guide the development of novel therapeutic strategies for prion diseases.

Dr. Fiona Houston

(The Roslin Institute, Royal Dick School of Veterinary Studies, University of Edinburgh)

Dr. Fiona Houston is a Group Leader in the Division of Immunology at the Roslin Institute, part of the Royal (Dick) School of Veterinary Studies at the University of Edinburgh. She has a veterinary degree, a PhD in bovine immunology, and in the past 25 years has led a number of large research projects on pathogenesis and transmission of ruminant prion diseases, mainly in sheep. Amongst the most significant findings were the first demonstration of efficient transmission of prions by blood transfusion, and experimental transmission of BSE to sheep previously considered to be genetically resistant to scrapie. Current research interests include blood-based prion diagnostic tests, application of in vitro prion replication and cell culture models to study genetic resistance and prion strain discrimination, and cross-species comparison of age-related neurodegeneration.

Prions in Blood: Lessons from a Large Animal Model

Prior to the emergence of variant Creutzfeldt-Jakob disease (vCJD) as a novel human prion disease, blood and blood products were considered to pose a low risk for disease transmission. Sheep experimentally infected with bovine spongiform encephalopathy (BSE) have provided a convenient model for studying transfusion transmission of vCJD. In particular, the similarity in size of sheep and humans offers an advantage over rodent models, by allowing collection of blood volumes and processing methods comparable to those used in transfusion medicine. Outcomes of preliminary experiments demonstrated surprisingly efficient transmission of infection by blood transfusion, the relevance of which was borne out by subsequent identification of transfusion-related vCJD cases. This talk will describe the challenges of establishing an experimental model of blood-borne prion infectivity in sheep, and its diverse applications, including study of risks associated with different blood components, efficacy of prion removal devices, development of preclinical diagnostic tests and biomarker discovery.

Dr. Joel Watts

(Tanz Centre for Research in Neurodegenerative Diseases and Department of Biochemistry, University of Toronto)

Dr. Watts obtained his PhD in Laboratory Medicine and Pathobiology from the University of Toronto and then conducted postdoctoral research in the laboratory of Nobel laureate Stanley Prusiner at the University of California San Francisco. He is currently a Principal Investigator at the Tanz Centre for Research in Neurodegenerative Diseases, an Associate Professor within the Department of Biochemistry at the University of Toronto, and is the Canada Research Chair in Protein Misfolding Disorders. His research interests include studying the role of self-propagating, prion-like protein aggregates in Alzheimer's disease and Parkinson's disease as well as exploiting the unique properties of the bank vole prion protein to develop improved animal and cellular models of the prion disorders.

α-Synuclein strains as drivers of disease heterogeneity

The defining feature of synucleinopathies such as Parkinson's disease (PD) and multiple system atrophy (MSA) is the presence of pathological α-synuclein aggregates within the brain. An emerging theory is that the progressive nature of PD and MSA stems from the formation and cell-to-cell spread of self-propagating α-synuclein aggregates. Additionally, evidence is accumulating that α-synuclein can polymerize into structurally distinct "strains" of aggregates, akin to the existence of different prion strains in the prion diseases. We and others have hypothesized that conformational strains of α-synuclein may be responsible for enciphering disease variability across PD, MSA, and related neurodegenerative disorders. Using a transgenic synucleinopathy mouse model, we have found that distinct disease phenotypes can be induced by injection with different strains of recombinant or human disease-derived α-synuclein aggregates. Recently, we have been investigating how different strains of α-synuclein aggregates may arise in the brain. We have found that considerable conformational heterogeneity exists between individual preparations of recombinant α-synuclein fibrils generated under identical conditions. Moreover, α-synuclein aggregates formed spontaneously in the brains of transgenic mice are conformationally diverse and can be classified into three distinct types. These results demonstrate that α-synuclein can spontaneously form multiple self-propagating strains within an identical molecular environment both in vitro and in vivo. This suggests that stochastic misfolding into distinct aggregate structures drives the emergence of α-synuclein strains.

Dr. Nina Oberbeck

(GATE Bioscience, California, United State of America)

Dr. Oberbeck is an accomplished translational biologist and a seasoned drug developer. She is the Senior Director of Translational Sciences at Gate Bioscience, where she leads the Prion program. Dr. Oberbeck earned her Ph.D. in Molecular Biology from the MRC Laboratory of Molecular Biology, University of Cambridge, where she investigated DNA repair mechanisms under the mentorship of Dr. K.J. Patel, FRS FMedSci. She also holds an MA (Hons) and MSci in Natural Sciences from Magdalene College, University of Cambridge. She completed her postdoctoral training at Genentech Inc. under Dr. Vishva Dixit, focusing on the signalling pathways that regulate epidermal stem cell differentiation. She has authored several first-author publications in leading scientific journals, including Nature and Molecular Cell. Dr. Oberbeck has presented Gate's work on prion disease internationally at the Prion 2024 meeting in Nanchang, China, and the 14th Annual National CJD Conference in Melbourne, Australia, in 2023.

Oral Small Molecules for Reducing Prion Protein Levels: A New Therapeutic Strategy for Prion Disease

Evidence from genetic models and antisense oligonucleotide (ASO) studies indicate that lowering prion protein (PrP) in the brain is therapeutically beneficial in prion disease. Molecular Gates are small molecules that selectively eliminate extracellular proteins inside the cell. Molecular Gates have a precise mechanism: they selectively block the interaction between a target protein's unique signal peptide and the secretory translocon through which the protein must pass to reach the cell surface. Blocked from export, the nascent target protein is degraded by the proteosome. This novel mechanism offers a new therapeutic approach for eliminating disease-causing proteins that are difficult to target with other modalities.

We are developing highly selective, orally bioavailable, and brain penetrant Molecular Gates that lower prion protein for the treatment of prion disease. We have synthesized and tested PrP-selective Molecular Gates and here we present data from one of these compounds, MG-813. MG-813 shows potent and complete prion-lowering in cell culture and substantial target engagement in the brain of mice dosed with compound. It also significantly extends the lifespan of prion-infected mice when administered chronically after symptom onset.

PrP-lowering ASOs are now being tested in the clinic. While promising, ASOs have limited potency related to their uneven distribution to the deep brain. In addition, long-term treatment requires invasive and repeat intrathecal dosing by lumbar puncture. By contrast, our novel mechanism paves the way for the development of a pill to treat or prevent prion disease.

Supporting Associations of prion diseases patients

APDP – Associação Portuguesa de Doenças Priónicas

The APDP – Portuguese Association for Prion Diseases – has the mission of supporting patients and families affected by prion diseases, providing emotional support, accessible information, and advocating for their rights. We work to raise awareness about these diseases, reduce stigma, and collaborate with researchers and healthcare professionals to drive scientific advancements and ensure equitable access to innovative diagnostics and treatments.

Webpage: www.prioes.pt

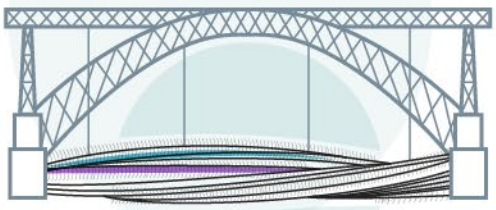
e-mail: geralapdp@gmail.com

Fundación Española de Enfermedades Priónicas (former Asociación Española de CJD)

Founded in 2013 as the Asociación Española de CJD, the organization aimed to support and connect affected individuals. With growing membership and visibility, it became the Fundación Española de Enfermedades Priónicas in 2020, expanding its efforts to provide scientific and medical guidance, psychological support, and legal counseling. Committed to advancing research, the foundation dedicates all its funds to financing projects aimed at developing effective therapies.

Webpage: https://fundacionprionicas.org/

e-mail: info@fundacionprionicas.org



Oral Communications

Prion Structure and Biology

Preclinical Development of Drug-Like Compounds Halting Prion Neurotoxicity

Emiliano Biasini

Department of Cellular, Computational & Integrative Biology (CIBIO), University of Trento, Italy

Prion diseases are rare and incurable neurodegenerative disorders affecting humans and animals and originating in sporadic, infectious, or genetic forms. All prion diseases share a common molecular mechanism: the conversion of the cellular prion protein (PrP) into an infectious form (PrPSc) that accumulates in the brain of affected individuals. PrP has a dual role in prion diseases by acting as a substrate for PrPSc propagation and transducing its neurotoxicity. The latter concept is exemplified by PrP molecules carrying mutations in the central region, which have been shown to spontaneously induce neurotoxicity by destabilizing the neuronal membrane. We previously employed a phenotypic assay to identify small molecules capable of blocking mutant PrP toxicity. A subsequent hit-to-lead campaign to enhance the potency, reduce cytotoxicity, and improve the pharmacokinetic properties of one of these compounds led to a series of analogues active in the low nanomolar range and possessing pharmacological properties suitable for in vivo use. SM compounds do not exert their effects by binding, re-localizing, or suppressing PrP. Efforts to identify the target of SM compounds by leveraging structural similarities with known molecules were unsuccessful. We thus initiated a chemoproteomic analysis in collaboration with the EU-OPENSCREEN consortium. An SM derivative was immobilized on magnetic beads to co-precipitate proteins interacting with the SM moiety, which were then dose-dependently eluted with two additional SM derivatives and identified by mass spectrometry. We found the RNA Binding Motif Protein 8A (RBM8A), a core component of the exon junction complex, as the most promising candidate. Preliminary analyses by nano DSF confirmed SM compounds' binding to RBM8A. We are currently conducting additional in vitro, cell-based, and in vivo experiments to assess the role of RBM8A in the toxicity of prion diseases and the rescuing ability of SM compounds. Our results lay the groundwork for translating the therapeutic potentials of SM compounds to clinical settings and provide potential mechanistic insights into prion neurotoxicity.

Structural basis for glycoform ratio specificity among prion strains

Francesca Peccati[1,2]

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[2]Ikerbasque, Basque Foundation for Science, 48013 Bilbao, Spain
fpeccati@cicbiogune.es

Glycoform ratios are intrinsic characteristics of prion strains, and the presence of glycans affects PrP^{Sc} propagation in a strain-dependent manner. As such, glycans are important determinants of strain fitness. Interpretation of glycoform preferences in terms of steric and electrostatic effects has long been hindered by the lack of detailed structural information distinguishing prion strains. In recent years, near-atomic resolution cryo-EM structures of prion fibrils have become available (e.g. Manka et al. 2022; Kraus et al. 2021). However, post-translational modifications such as glycosylation remain invisible to structural techniques due to their heterogeneity and flexibility. Molecular modeling can fill this gap by providing complete atomistic models of prion fibrils.

In this contribution, we use microsecond-scale, all-atom periodic molecular dynamics simulations to characterize the dynamic behavior of distinct glycosylation patterns in different prion strains (Figure). We examine atomic-level contacts between glycans on consecutive fibril strands, characterizing glycan–glycan and glycan–protein interactions, with particular emphasis on the role of sialylation in modulating key steric and electrostatic contacts. We show how glycosylation modulates the flexibility of fibrillar aggregates in a strain-specific manner, providing a molecular basis for the glycoform preferences observed in prion strains RML and 263K.

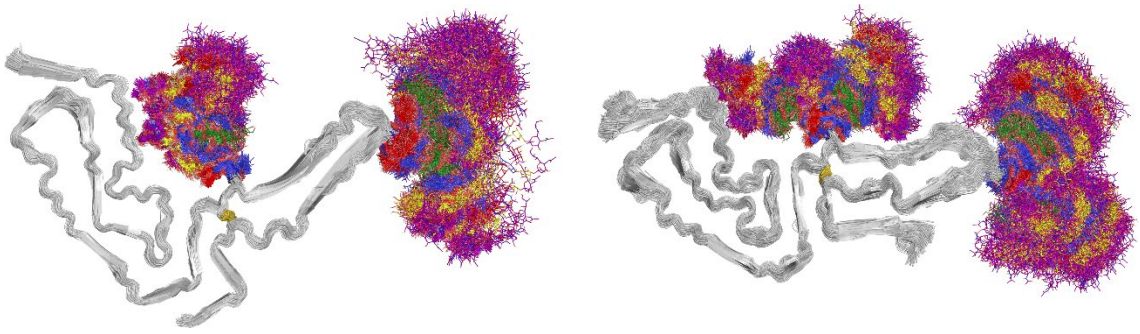

Figure Overlay from molecular dynamics simulations of glycosylated prion fibrils corresponding to strains RML (left) and 263K (right).

Structural Dynamics and Kinetic Interplay in Prion Assembly

Raul Cacheiro Vazquez[2]; Mathieu Mezache; Jeremie Mathurin[3]; Angelique Igel[1], Ariane Deniset-Besseau[3]; Davy Martin[1], Pierre Sibille[1] Jesus Requena[2] and Vincent Béringue[1] and Human Rezaei[1]

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[2]CIMUS Biomedical Research Institute & Department of Medical Science. University of Santiago de Compostela-IDIS, Spain
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Corresponding author: raul.cacheiro.vazquez@usc.es

The prion pathology is based on autonomous structural information propagation towards single or multiple protein conformational changes. Since this last decade the prion concept referring to the transmission of structural information has been extended to several regulation systems and pathologies including Alzheimer and Parkinson's diseases. The unified theory in Prion replication implies structural information transference from the prion to a non-prion conformer through a mechanism also called improperly, with regards to biophysical considerations "seeding" phenomenon. Recently we reported that prion replication is intrinsically source of structural diversification. The coexistence of multiple prion assemblies with different structure and replication propensity questions how they are maintained within the same media and how they escape to best replicator selection.

Through the analysis of the dynamics of the quaternary structure of prion assemblies—both infectious synthetic (mouse and bank vole) and brain-derived—we demonstrated the existence of an exchange process within structurally diverse prion assemblies at the single-assembly level, using nanoscale infrared spectroscopy (NanoIR) and dynamic atomic force microscopy. Additionally, the structural characterization of infectious synthetic prion assemblies by cryo-electron microscopy has been initiated.

This exchange process results in the appearance of damped oscillations in prion replication kinetics. Data assimilation and kinetic modelling led us to propose a kinetic scheme in which structurally distinct prion assemblies catalytically exchange material. According to this model, a catalytic depolymerization step competes with a catalytic conformational conversion.

Spontaneous neurodegenerative disease in a transgenic mouse model expressing H95Y PrP-amino acid change

Juan-Carlos Espinosa[1]*, Juan-María Torres[1], Alba Marín-Moreno[1], Sara Canoyra[1], Anna Burato[2], Arianna Ciullini[3], Chiara Maria Giulia De Luca[4], Edoardo Bistaffa[4], Fabio Moda[3,5], and Giuseppe Legname[2,6]

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[2] Laboratory of Prion Biology, Department of Neuroscience, Scuola Internazionale Superiore di Studi Avanzati (SISSA), Trieste, Italy
[3] Unit of Laboratory of Medicine – Laboratory of Clinical Pathology, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy
[4] Division of Neurology 5 and Neuropathology, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy
[5] Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy
[6] ELETTRA Sincrotrone Trieste S.C.p.A, Basovizza, 34149 Trieste, Italy.
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Prion protein has long been known as a copper-binding protein via histidine residues in the octapeptide repeats (OR) and the non-OR region located in the disordered N-terminal tail of the protein. The functional implications of copper binding to PrP are unclear, but copper seems to play an essential role in prion disease. Prion diseases are caused by a conformational change in the prion protein from its cellular form (PrP^{C}) to a misfolded isoform (PrP^{Sc}).

Transgenic mouse lines expressing the mouse prion protein replacing with a histidine to tyrosine (H95Y) substitution at codon 95, disrupting the non-OR copper-binding site, have been generated and characterised.

Transgenic mice overexpressing approximately two fold PrP H95Y developed clinical signs and died at around 90-100 days respectively, exhibiting spongiform degeneration. Brain PrP^{res} in these mice resembled that of atypical/Nor98 scrapie. Inoculation of brain homogenates from terminally ill mice PrP H95Y overexpressing mice into mice expressing low levels of PrP H95Y (one fold) or into Tga20 mice also induced lethal, spongiform encephalopathy, presenting the same atypical scrapie-like PrP^{res} pattern.

The H95Y substitution in the non-OR region may promote PrP^{C} to PrP^{Sc} conversion, leading to a spontaneous, rapidly progressing, and transmissible neurodegenerative disease in transgenic mice. This finding suggests a critical role for the non-OR copper-binding site in prion disease. Transgenic mice overexpressing approximately two-fold PrP H95Y can be considered a useful model for spontaneous prion disease studies.

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Unfold to refold: exploring the early events of PrP^{C} to PrP^{Sc} conversion with solution NMR and Molecular Dynamics

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It might have been expected that elucidation of the PrP^{Sc} structure would allow an almost immediate understanding of its propagation mechanism. However, PrP^{Sc} is, disappointingly, a "regular" amyloid: it can just act as a passive template of a previously unfolded protein. Then, how is the PrP^{C} ~121-231 folded domain (FD) converted to the PrP^{Sc} conformation? It necessarily has to unfold before it can fit into the PrP^{Sc} template, that might be a relatively passive actor in this process. PrP^{Sc} might have no other role than trapping unfolded stretches of FD as they become available. However, PrP^{Sc} does play a key role in trapping/templating the ~90-120 N-terminal unfolded tail of PrP^{C}, tethering the FD to the templating surface.

To study the spontaneous FD unfolding process, we performed thermal unfolding of recombinant bank vole 90-231 PrP^{C} and tracked early changes using a suite of solution NMR experiments. In contrast with previous experiments, we are interested in the early events only, not in a complete conversion to a non-PrP^{Sc} amyloid. We identify the β-strand 1 and its contiguous loops as the regions with a higher propensity to unfold first, and part of α-helix 2 and β-strand 2 as the most resilient conformational elements. Molecular Dynamics simulations identify separation of α-helix 1 from the FD ensemble as another key intermediate event during unfolding. These findings allow establishing a tentative timeline of early FD unfolding. Our ultimate aim is fitting these data in a mixed experimental/modelling strategy to build an atomistic model of PrP^{Sc} propagation.

Self-Organized Spatial Patterns of Prion Replication in Organotypic Cerebellar Slices Infected with the 127S Strain

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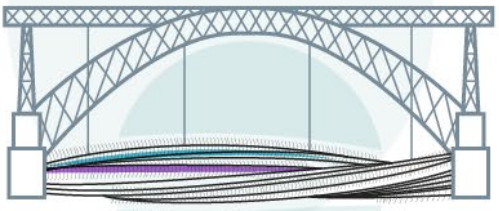
Prion diseases are associated with the misfolding and propagation of the pathological isoform of the prion protein (PrP^{Sc}), yet the mechanisms governing their spatial dynamics in neural tissues remain poorly understood. In this study, we used an *ex vivo* model using long-term organotypic cultures of murine cerebellar slices to investigate the replication dynamics of the ovine 127S prion strain.

We demonstrated that this model supports dose-dependent PrP^{Sc} accumulation over a wide dynamic range, detectable via histoblotting. Infection initiated immediately after culture setup leads to the emergence of complex and dynamic spatial patterns of PrP^{Sc} deposition. These patterns evolve over time and are modulated by both cerebellar microanatomy and the infection protocol, suggesting that both tissue structure and inoculation mode contribute to the observed distribution profiles.

In light of our previously published results on prion replication dynamics, this work points to a self-organized, non-linear reaction-diffusion process underlying prion replication in this model. These findings provide novel insights into prion neuroinvasion and open avenues for spatially resolved modeling of prion dynamics.



Oral Communications

Prion Diseases in animals

Gene therapy for prion diseases using an adeno-associated viral vector

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Developing a treatment for prion diseases remains a significant challenge, as classical drug therapy has been proven ineffective. We propose an alternative treatment using gene therapy. Specifically, we aim to introduce a mutation that confers dominant-negative resistance to prion protein misfolding, described in humans, via an adeno-associated virus type 9 (AAV9) vector, which can efficiently cross the blood-brain barrier. This mutation consists of replacing glycine (G) with valine (V) at codon 130 of the ovine *PRNP* sequence.

For this study, prion-infected Tg338 mice were treated with the AAV9 vector through different routes and using different treatment regimens. These regimens included: a single dose administered 7 days post-inoculation, a single dose at 30 days post-inoculation, two doses given at 7 and 14 days post-inoculation and three doses administered at 7, 14, and 21 days post-inoculation. Additionally, the corresponding positive controls for each group were inoculated. Upon reaching the terminal disease stage mouse brains were analyzed via western blot and immunohistochemistry.

Preliminary results suggest a survival extension of 6,23% to 47,29% in treated animals when compared with their respective controls. Greatest survival period extension was detected in mice orally infected and intravenously treated with 2 doses. However, several mice in the group treated with 3 doses have not developed disease to this day (day 129 post-inoculation). Neuroinflammation and prion deposition analysis show no significant differences between groups.

Synthetic Cervid Prions in Gt Mice as a Model for Novel CWD Strain Emergence and Evolution

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The characterization of chronic wasting disease (CWD) cases in Northern Europe as distinct prion strains from those in North America has raised concerns about their zoonotic potential and the possible emergence of new strains in the future. Based on the hypothesis of the idiopathic/spontaneous origin of some Nordic strains, we used Protein Misfolding Shaking Amplification (PMSA) to generate a diversity of synthetic cervid prions through the spontaneous misfolding of recombinant prion proteins (rec-PrP). These synthetic prions serve as ideal models, enabling us to explore the potential strain diversity arising from spontaneous misfolding of cervid PrP.

We selected six of the resulting ensembles of conformers—three based on deer PrP and three on elk PrP—with two from each group generated in the presence of dextran sulfate and one in the absence of any cofactor. After demonstrating their ability to induce prion disease in transgenic mice expressing bank vole PrP, we further characterized them through intracerebral and intraperitoneal inoculation into cervidized gene-targeted mice, a model that better recapitulates natural cervid strain properties.

Interestingly, we observed important differences in the outcome of the bioassays, particularly depending on the genotype of the inoculum and the route of inoculation. We analyzed the properties of the resulting samples post-inoculation through various *in vitro* approaches, finding that, despite their heterogeneous properties, some cervid synthetic prions exhibit characteristics resembling those of certain Nordic moose CWD strains, while others present unique aspects, highlighting the high variability that the emergence of new CWD strains could entail.

Different transmissible properties of CWD prions from North America and Northern Europe in cervidized gene-targeted mice

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Chronic wasting disease (CWD) from North America (NA) transmits through multiple infection routes in the natural host. However, less is known about the transmissible properties of newly emergente Nordic CWD in reindeer (R-NO) and moose (M-NO). Our group developed gene-targeted (Gt) mouse models that express the cervid prion protein (PrP) under the endogenous regulatory elements of the murine *Prnp* promotor. Consequently, these Gt mice express exogenous PrP at physiological levels in the periphery and replicate NA CWD in several extraneural tissues. Utilizing these mice, we found that while intraperitoneal and oral challenge of NA CWD retain native strain properties, intracerebral inoculations resulted in altered CWD prions producing a distinct disease outcome. These data suggest that there are tissue-specific cofactors responsible for prion strain selection, replication, and propagation. Based on these findings, we transmitted several NA CWD and NO CWD isolates by nine different routes of inoculation to our Gt models. Interestingly, while NA CWD is highly competent at transmitting by all routes of inoculation, R-NO and M-NO CWD prions are less efficient. These data further support the hypothesis that NA CWD harbor distinct strain properties from those comprising emergent R-NO and M-NO CWD isolates. These transmissible differences have major public health implications for the ongoing CWD outbreaks in Northern Europe. Moreover, these findings of variable transmission dynamics suggest that their distinct strain properties alter their capacity to replicate in peripheral compartments and bolster our previous findings of tissue-specific cofactors responsible for prion strain selection and propagation.

Remarkable diversity of emergent CWD strains in Swedish moose

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Chronic wasting disease (CWD), a highly infectious prion disease of deer, elk, moose, and other cervids, poses an increasingly unpredictable threat to human health. While the CWD epidemic has existed in North America for decades, CWD recently emerged in Norway, Sweden and Finland. Our previous studies showed that the strain properties of CWD prions from Norwegian and Finnish cervids were distinct from North American CWD. Methods: To characterize Swedish moose CWD isolates, we performed transmission studies on the four reported moose cases in our gene targeted (Gt) and other mouse models. We analyzed the kinetics of disease, PrP^{Sc} accumulation in the brain, neuropathology, prion conformational stability and assessed in vitro amplification properties by RT-QuIC and protein misfolding cyclic amplification (PMCA). Results:

We found that Swedish moose CWD isolates have diverse transmission kinetics, including one disease isolate displaying a remarkably rapid onset of disease in only 90 days. Conformational stability, histological features and amplification properties differed among the Swedish isolates and were distinct from North American and Norwegian CWD. Conclusions: The strain properties of CWD prions in Nordic countries are highly diverse and their capacity for adaptation is unpredictable.

Zoonotic potential of chronic wasting disease prions demonstrated by passage in non-human primates and rodents

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Chronic wasting disease (CWD), a fatal prion disease affecting cervids, is spreading across North America. CWD prions persist in the environment, are shed in excreta and accumulate in peripheral tissues, including muscle, of infected cervids, raising concerns about its zoonotic potential. Although epidemiological studies in CWD-endemic regions did not show an increase in prion diseases among humans, experimental studies suggest that CWD prions can adapt to new hosts, potentially lowering transmission barriers. Using *Cynomolgus* macaques, as a model, we explored the zoonotic potential of CWD by inoculating macaques with cervid-derived CWD prions. Although most macaques remained asymptomatic, some displayed neurological signs typical of prion disease. Sensitive *in vitro* prion amplification assays revealed low levels of prion seeding in macaque tissues, in the absence of detectable protease-resistant prion protein. Inoculation of transgenic cervidized mice and bank voles with tissue homogenates from macaques induced prion disease, achieving 100% transmission rates upon serial passage in bank voles. Biochemical and histopathological analysis confirmed classical prion characteristics in bank voles, with unprecedented tissue tropism in the intestinal tissues. These findings reveal that CWD prions retain infectivity across species and emphasize that primate infection may manifest subclinically and atypically while still enabling secondary transmission. Our results challenge earlier conclusions that minimized the zoonotic risk of CWD and underscore the need for continued surveillance. Given the expanding prevalence of CWD in wild and farmed cervids, these findings highlight a latent zoonotic threat that warrants serious consideration and further research to evaluate the risks of human exposure.

Transmission of reindeer chronic wasting disease to sheep

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Chronic wasting disease (CWD) is a prion disease affecting cervids, first described in USA in 1967. In 2016, the first European case was detected in reindeer (*Rangifer tarandus*) in the Nordfjella mountains in Norway. Reindeer CWD is contagious, and prions are present in the nervous system and peripheral tissues.

CWD has been detected in American wild pigs, and because Nordfjella is a summer pasture for more than 60.000 sheep there is a concern for spillover to sheep. American strains have been experimentally transmitted to sheep perorally and intracerebrally. *In vitro* and rodent strain typing studies demonstrate that American and Norwegian isolates are of different strains.

Six lambs of the VRQ/VRQ scrapie susceptible genotype were intracerebrally inoculated with reindeer CWD. During the study, the animals were regularly examined neurologically and rectal biopsies sampled. At the study endpoint, the animals were euthanized, and tissues examined with conventional diagnostic methods (histopathology, immunohistochemistry, western blot (WB) and ELISA), and ultrasensitive methods (Protein Misfolding Cyclic Amplification (PMCA) and Real Time Quaking Induced Conversion (RT-QuIC).

By ultrasensitive methods, all but one sheep had amplification of prions in the central nervous system. Prions were further amplified from several lymph nodes of all sheep. Three lymph nodes were ELISA and WB positive. This study demonstrates that reindeer CWD is transmissible to sheep by the intracerebral route, although with minimal detection of prions.

We also inoculated six newborn lambs perorally with reindeer CWD, and some preliminary results from this parallel study will be presented at the conference.

Generation of *bona fide* Mongolian gerbil (*Meriones unguiculatus*) prions *in vitro*

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The Protein Misfolding Shaking Amplification (PMSA) technique was employed to misfold a variety of recombinant mammalian prion proteins *in vitro*. For the Mongolian gerbil (*Meriones unguiculatus*), we obtained a single misfolded conformer that successfully converted both TgVole and gerbil PrP^{C} into PrP^{res} in normal brain homogenate-based PMCA reactions. These gerbil prions demonstrated infectivity upon bioassay in TgVole(I109)1x mice while showing no transmission to humanized Tg340 mice. To validate PMSA as a system capable of generating authentic prions beyond laboratory mice, we challenged the Mongolian gerbil model with recombinant gerbil prions through two routes: directly from PMSA (non-glycosylated and lacking the GPI anchor) and after adaptation through PMCA using gerbil normal brain homogenate as substrate (providing adaptation to a more natural PrP^{C} source with complete post-translational modifications). Both inocula successfully transmitted disease following intracerebral inoculation, with an incubation period of 540-565 days.

We present a comprehensive biochemical and neuropathological characterization of this bioassay, demonstrating the successful reproduction of transmissible spongiform encephalopathy in a species with no previous record of naturally occurring prion disease. These findings validate PMSA as an effective method for generating substantial quantities of authentic prions suitable for structural studies and highlight its utility in investigating prion disease biology in previously unexplored species.

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Case-control studies in scrapie flocks and recent findings on protective prion protein genotypes in Iceland.

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Although used in Europe for many years, breeding for scrapie resistance in Iceland could not be adopted until recently after a widespread search for scrapie protective prion protein variants in Iceland resulted in the first few findings of Icelandic sheep carrying ARR. Furthermore, $AT_{137}RQ$, shown to be protective against scrapie in Sarda sheep in Italy, was found on several farms.

A case-control study has been performed on 18 Icelandic scrapie flocks detected in the period between 1997 and 2024 and originating from six different movement protection zones. The purpose of the study was to access the influence of possibly protective genotypes in Icelandic sheep. Total number of sheep analysed was 4978 of which 406 tested positive for scrapie. Total number of 20 different PRNP genotypes were detected, when polymorphism at codons 137, 138 and 151 was considered in addition to 136, 154 and 171. Among the observed genotypes with a significant lower scrapie susceptibility compared to the wild type (ARQ/ARQ) were AC_{151}/ARQ, AHQ/ARQ and AHQ/VRQ. No scrapie-positive sheep was identified which carried T137; however, the frequency of genotypes with T137 in scrapie-affected flocks was too low for statistical validation.

A breeding program using ARR is already in use and based on the results of this case-control study, additional selection on three more alleles, $AT_{137}RQ$, $AC_{151}RQ$ and AHQ, is recommended to assist in keeping the diversity of the sheep population and possibly provide protection against unforeseen changes in the scrapie infectious agent in the future.

Eradication of scrapie in a goat herd by breeding for resistance

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Eradication of classical scrapie (CS) by breeding for resistance in goats is challenging because of the rarity of resistant genotypes, and its success in herds with naturally occurring scrapie is unknown. This study describes the outcome of a breeding program in a CS-affected dairy goat herd to replace susceptible goats (prion protein genotype *QQ222*) in favour of more resistant goats (*QK222 or KK222*).

In 2012, genotyping of 1,567 breeding adults detected two male (17%) and 78 female (5%) *QK222* goats. The objective was to increase the resistant population by generating *KK222* males to be exclusively used for breeding whilst keeping females until the end of their productive life regardless of genotype.

Between 2012-2023, 5,143 goats over 18 months sent for slaughter or fallen stock were tested for CS by rapid screening test, of which 33 *QQ222* were positive. Peripheral tissues were also examined in a subset of 86 *K222* and 48 *QQ222* goats, which did not detect more scrapie cases. CS was last confirmed in 2020; 80 *QQ222* goats remained when compulsory monitoring stopped, of which 53 have been culled or died with no evidence of infection by brain and peripheral tissue examination.

Eradication of CS may be possible by breeding for resistance. There was no evidence CS would have been missed if only the screening test was used for diagnosis or that *K222* goats were infected. This is the first study demonstrating the effect of selective breeding on scrapie eradication in a herd with naturally occurring CS.

Co-presence of classical scrapie 22A but not classical BSE in dutch sheep with atypical scrapie

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Nineteen cases of atypical scrapie (AS) have been detected in the Netherlands since the beginning of the active TSE surveillance in 2001. We inoculated brain and tonsillar tissue from the first 2 AS cases (one AHQ/AHQ and one AFRQ/AFRQ sheep) into wild type and (b)ovine PrP transgenic mice.

Transmission was only seen in the ovine PrP transgenic mice inoculated with brain tissue but not with tonsillar tissue. Incubation periods (IP) of AS in the ovine VRQ transgenic tg338 mice stabilized at 3rd or 4th passage at around 240 days post inoculation (dpi). No stabilization of the IP was seen in the ovine ARQ transgenic tg59 mice even after 4 passages. Cross passage between tg338 and tg59 mice confirmed that a single, identical strain was isolated in these 2 mouse lines.

At the primary passage of AS from the AHQ/AHQ sheep in tg338, 2 mice died with a much shorter IP (110 dpi). The PrP^{res} profile of these mice showed a classical scrapie pattern with a mixed PrP^{Sc} profile. Secondary transmission from these mice into VM and tg338 mice showed the co-presence of the classical scrapie strain 22A with AS.

No transmission was seen in the wild type RIII and VM mice or the bovine PrP transgenic tg110 mice. Blind secondary transmission of a brain pool made from the brains of the tg110 mice of the first passage into another panel of tg110 mice was also negative. We can thus not confirm the previously reported co-presence of classical BSE with AS.

Prion evolution of Nor98/Atypical Scrapie in a homologous ovine PrP^C Context

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Nor98/Atypical scrapie (AS) is a prion disease that occurs sporadically in sheep and goats. Previous studies have shown that AS transmission to heterologous species can lead to the emergence of classical PrPres prion strains, such as classical BSE-like in cattle and classical scrapie-like prions in bank voles. Although the emergence of different scrapie strains during intra-species transmission has been reported, the potential evolution of AS prion mechanisms within the same species remains unclear.

To investigate this, we studied the transmission of a diverse panel of AS isolates from sheep and goats, differing in genotype and geographical origin, in a homologous ovine PrP context. Using ARQ-OvPrP-

Tg501 mice, we conducted both *in vivo* and *in vitro* PMCA analyses. The isolates efficiently infected ARQ-OvPrP-Tg501 mice, showing a complete attack rate and homogeneous survival times. Notably, some transmissions resulted in the emergence of distinct prion strains, including 19kDa (BSE-like), 21kDa (classical scrapie-like), and mixtures of these agents. Propagation by PMCA in ARQ-OvPrP-Tg501 substrate also showed the emergence of classical PrPres prions.

The emergence of classical prions through the transmission of AS in the ovine PrP could be caused by the coexistence of strains in the isolate or the evolution of the AS through propagation in the ovine PrP. This supports the hypothesis that atypical prions may serve as a source of prion diversity with a tendency to evolve into classical forms. Understanding this process is crucial for assessing the potential risks of AS transmission in livestock and its implications for prion disease control.

Funding information:

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Large-scale association study on the combined effect of genetics and age on atypical scrapie risk in the Portuguese sheep population.

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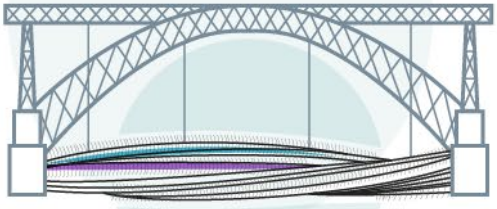
Atypical Scrapie (AS) accounts for a significant proportion of small-ruminant TSE cases in the EU, with Portugal showing a notably high prevalence (~10 cases per 10,000 tested animals). Unlike classical scrapie, AS is considered non-contagious, primarily influenced by age and PRNP gene polymorphisms, especially at codons 136, 154, 171, and uniquely, 141. This study leverages the largest national AS dataset in Europe to investigate the association of 4-codon PRNP polymorphisms with disease risk in Portuguese sheep and to assess the combined effect of genotype and age.

We compared 4,320 sheep randomly genotyped between 2010 and 2017 with 667 confirmed AS cases detected from 2003 to 2023. Multivariable fixed- and mixed-effects Poisson regression models were applied, including year of collection, age (in months), and, sequentially, 4-codon haplotype and genotype as covariates. The potential for age to act as an effect modifier on genetic susceptibility was also assessed.

Among the most common haplotypes, AFRQ showed the highest age- and year-adjusted excess risk compared to the ALRQ wild type, followed by ALHQ, ALRR, and ALRH. At the genotype level, the greatest risk increases relative to ALRQ/ALRQ were seen with AFRQ/ALHQ, followed by AFRQ/AFRQ, ALHQ/ALHQ, AFRQ/ALRH, and AFRQ/ALRR. Conversely, the VRLQ haplotype and the ALRQ/VRLQ genotype were associated with the lowest risk. A clear effect modification, rather than confounding, was observed between age and genotype.

This unprecedented large-scale study not only confirms prior findings but also provides new insights into the epidemiology of Atypical Scrapie.



Oral Communications

Prion Diseases and Prion-Like diseases in humans

HeliCure targets α-synuclein oligomers and restores motor function in a preclinical mouse model of Parkinson's disease.

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder worldwide and remains incurable despite extensive research. PD is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta, leading to movement-related symptoms such as rigidity, tremor, or bradykinesia. The major pathological hallmark of PD is the accumulation of protein inclusions in the brain, named Lewy bodies and Lewy neurites, primarily composed of aggregated α-Synuclein (αSyn). Of all species formed during amyloid aggregation, αSyn oligomers are considered the main culprits for neuronal homeostasis collapse and disease propagation throughout the brain. Toxic αSyn oligomers present hydrophobic and anionic surfaces, distinguishing them from αSyn monomers. We have exploited these differential structural properties to identify a family of peptides that bind to these αSyn species with low nanomolar affinity, without interfering with the monomeric functional protein. With a structure-function relationship in hand, we identified a human neuropeptide, HeliCure, that showed remarkable anti-aggregation activity in vitro. Importantly, we tested HeliCure's effect in a PD mouse model overexpressing human αSyn in dopaminergic neurons using a non-invasive administration route. Behavioral assessment showed a re-establishment of motor coordination and significant improvements in motor skills and balance after HeliCure treatment, recovering healthy control levels. Overall, these findings highlight HeliCure's potential to overcome current therapy limitations by distinguishing between functional and pathological αSyn forms, paving the way for a promising disease-modifying treatment for PD. Its success could mark a significant breakthrough in this challenging field, impacting the lives of millions of patients.

Advancing prion diagnostics: Novel RT-QuIC substrate enables detection in tear fluid and enhances cerebrospinal fluid sensitivity

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Prion diseases (PD) are fatal neurodegenerative disorders that require sensitive and specific diagnostic methods for timely detection. Seeded aggregation assays, such as real-time quaking-induced conversion (RT-QuIC), have advanced detection by amplifying minuscule amounts of misfolded prion protein scrapie (PrP^{Sc}) in cerebrospinal fluid (CSF) and other matrices. We recently demonstrated PrP^{Sc} detection in tear fluid (TF) using a modified protocol, establishing TF as a promising non-invasive diagnostic biofluid. This study refined RT-QuIC with novel recombinant human prion protein (PrP) substrates in CSF and TF. Full-length Human E200K (FL Hu E200K) substrate enhanced diagnostic sensitivity for Creutzfeldt-Jakob disease (CJD) and genetic PD (gPD), increasing rates from 78% to 93% in CJD, and 20% to 77% in gPD. TF RT-QuIC achieved diagnostic sensitivity of 84% for sCJD and 70% for gPD. These findings highlight TF as a practical alternative to CSF for biomarker development in prion diseases and other neurodegenerative disorders.

PrionPro: An International Quality Control Program for Prion Disease Biomarkers

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We are pleased to announce the launch of PrionPro, the Prion Disease Biomarker Quality Control Program, a coordinated international initiative aimed at strengthening diagnostic accuracy and standardization in the field of prion diseases.

This program brings together 18 partner institutions across multiple countries, forming a collaborative network dedicated to the assessment and validation of key biomarker assays. As part of PrionPro, two major ring trials are currently underway: one focused on 14-3-3 protein detection, and the other on the Real-Time Quaking-Induced Conversion (RT-QuIC) assay.

These assays are essential tools in the antemortem diagnosis of prion diseases, yet variability in performance across laboratories remains a critical challenge. Through blinded sample distribution, and comprehensive performance analysis, PrionPro aims to evaluate inter-laboratory consistency, identify sources of variability, and establish a framework for ongoing quality assurance.

The outcomes of PrionPro will enhance confidence in biomarker-based testing, and contribute to broader efforts in the standardization of prion disease diagnostics.

This initiative marks a significant step toward international harmonization in prion disease diagnostics and sets a precedent for collaborative biomarker quality control programs.

Host-dependent variability in amyloid-beta propagation and deposition

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Alzheimer's disease (AD) is pathologically characterized by the deposition of misfolded amyloid-beta (Aβ) and tau proteins in the brain. Compelling data show that misfolded Aβ spreads in a manner akin to infectious prions. Noteworthy, other prion-like characteristics have also been described for these preteinacious aggregates. These include their ability to accelerate brain pathology upon administration in susceptible hosts, and the acquisition of different conformational variants, namely strains. Growing evidence highlights the heterogenous clinical manifestations and pathological features across AD individuals. This variability is attributed to the presence of distinct conformational strains of Aβ. In this study, we investigated the variability in Aβ-misfolding propagation of human and mouse isolates intracerebrally inoculated in different animal models of amyloid pathology (APP/PS1 or Tg2576 mice). Our histological evaluations show that the same inoculum induced different patterns of Aβ pathology in the cortex and hippocampus of Tg2576 and APP/PS1 mice, supporting the idea that both, seeds and hosts, are responsible to influence amyloid pathology. Moreover, we also observed that the Aβ seeds present in different brain regions of the same patient display different seeding capacities. This study shows that different hosts react differently to the same Aβ sources. Our data also suggest that the type of Aβ seeds varies in different brain regions of the same individual. Investigating the pathological significance of Aβ strains may be relevant for the future development of personalized diagnostic and treatments approaches.

Tracking disease progression in fatal familial insomnia: Longitudinal plasma biomarker analysis

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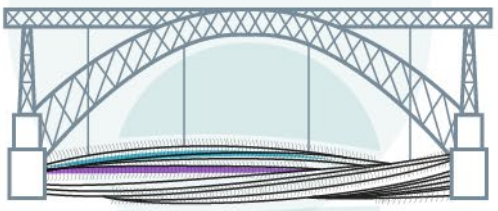
Fatal familial insomnia (FFI) is a genetic prion disease linked to the D178N-129M *PRNP* mutation. This disease manifests unpredictably during adulthood, leading to death within two years from symptom onset. The absence of prodromal biomarkers delays diagnosis until neurological damage is irreversible. This study aimed to identify plasma biomarkers for early diagnosis and prognosis. Longitudinal plasma samples were collected from 48 individuals: 22 non-carrier controls and 26 FFI mutation carriers, including 10 who converted from asymptomatic to symptomatic stages.

We measured neurofilament light chain (NfL), previously shown to increase presymptomatically in some slow-progressing genetic prion diseases, and five additional proteins: MMP9 (blood-brain barrier disruption), PPIA (neuroinflammation), α-synuclein (α-syn, synaptic function), YKL-40 (glial activation), and brain-derived tau (BD-tau, neurodegeneration). A subset of samples was also analyzed with the NULISAseq CNS Panel 120, a multiplex assay for CNS proteins.

NfL levels rose sharply at symptom onset in converters and continued to increase in the symptomatic phase. Although higher NfL levels were also observed in asymptomatic carriers vs. controls, variability limited its utility as a prodromal biomarker. Other markers (MMP9, α-syn, PPIA, YKL-40, BD-tau) showed inconsistent changes. NULISA analysis confirmed NfL elevation and identified 14 differentially expressed proteins in carriers vs. controls, such as GFAP, tau, and neurogranin, which were elevated only during the symptomatic phase.

In conclusion, while several markers showed promise for disease detection and progression monitoring, none reliably identified the prodromal phase, underscoring the need for more sensitive early biomarkers in FFI.



Scientific Posters

Prion Structure and Biology

Effect of Cervid Prion Protein Polymorphisms on Flexibility, Stability, and Spontaneous Misfolding of the Globular Isoform

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Protein misfolding is a key factor in proteinopathies, with certain polymorphisms influencing this process, sometimes increasing the susceptibility to the disorders and other times providing resistance. However, the precise mechanisms in which these polymorphisms affect protein integrity and how they contribute to misfolding propensity remain unclear. In the case of prion proteinopathies, they are caused by prions, the misfolded isoforms of the cellular prion protein (PrP^C). Among these, Chronic Wasting Disease (CWD) affects both free-ranging and captive cervids and can exhibit lymphotropic properties, making it the most widespread proteinopathy. Due to this broad occurrence, cervid PrPs and their polymorphisms have been extensively studied. To better understand the role of these polymorphisms, we analyzed 45 cervid PrP variants, encompassing all known polymorphisms in the central region of the protein, to assess their effects on flexibility, stability, and spontaneous misfolding propensity.

The cervid variants were expressed as recombinant PrP in *E. coli*, and their melting temperature was determined with circular dichroism to assess thermal stability. Additionally, the rec-PrPs served as substrates for Protein Misfolding Shaking Amplification (PMSA), enabling the assessment of each variant's spontaneous misfolding propensity. This process led to the formation of *bona fide* prions, as confirmed by inoculation of one of the resulting conformers into transgenic mice. In parallel, molecular dynamics simulations were conducted to analyze the structural flexibility of the variants. While differences in protein flexibility were observed, no correlation was detected among flexibility, thermal stability, and spontaneous misfolding propensity, suggesting that these properties are independent parameters.

Rabbit PrP Shows Reduced Aggregation in Cofactor-Driven Conversion Models

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Prion diseases are fatal neurodegenerative disorders associated with the misfolding and aggregation of the cellular prion protein (PrP). Interestingly, species like rabbits exhibit natural resistance to prion infection despite high sequence similarity to susceptible species such as mice. Understanding the molecular mechanisms underlying this resistance can provide valuable insights for therapeutic strategies and interspecies transmission control. In this study, we compared the aggregation behavior of recombinant rabbit and murine PrP in the presence of biologically relevant cofactors—glycosaminoglycans (heparin and dermatan sulfate), phosphatidic acid, and DNA oligonucleotides—using light scattering and fluorescence spectroscopy. While all cofactors induced aggregation, rabbit PrP consistently exhibited significantly lower aggregation, especially under acidic conditions. Notably, distinct aggregation mechanisms were observed: glycosaminoglycans promoted liquid–liquid phase separation (LLPS) leading to dynamic condensate formation, whereas lipids induced the formation of structured amyloid-like aggregates. These findings suggest that the lower susceptibility of rabbit PrP to conversion involves multiple protective mechanisms depending on the type of cofactor. Importantly, differences in aggregation were not directly correlated with binding affinity, indicating that intrinsic structural features, such as conformational stability and the contribution of the N-terminal domain, play a key role in modulating conversion susceptibility. These results support the use of rabbit PrP as a model to explore resistance to prion propagation and highlight cofactor-specific aggregation pathways as potential therapeutic targets. Overall, this study enhances our understanding of species barriers in prion diseases and opens new avenues for the development of anti-aggregation strategies.

Rapid generation of in vivo prion propagation models using AAV-delivered PrP variants in knockout mice

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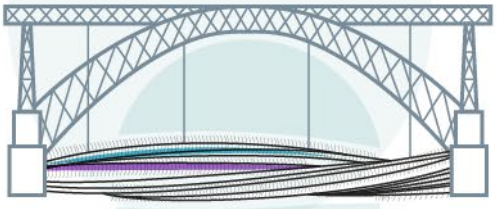
The study of prion biology has traditionally relied on transgenic mouse models, which, while valuable, require significant time and resources to develop. Here, we present a rapid and flexible alternative using adeno-associated virus (AAV) vectors to express modified prion proteins in PrP-knockout mice. We systematically evaluated several AAV vectors and optimized construct design by comparing central nervous system-specific promoters and regulatory elements. We selected a construct containing the human synapsin promoter, the MVM intron (from the minute virus of mice), and WPRE (Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element) to drive neuron-specific expression of mouse PrP carrying an L42-detectable epitope. The AAV genome was packaged in an AAV9P31 capsid capable of crossing the blood-brain barrier following systemic injection. Intravenous delivery of 1×10^{11} vg per mouse resulted in stable, widespread brain expression at levels comparable to or exceeding endogenous PrP. Upon challenge with mouse-adapted RML prions, AAV-PrP-injected mice developed characteristic signs of prion disease with accelerated kinetics (58–106 days post-inoculation), along with biochemical and histopathological features typical of RML infection. Serial transmission to wild-type mice is currently underway to confirm preservation of RML strain properties and validate prion propagation in this model. This system offers a versatile platform for generating and studying prion variants in an authentic brain environment, with particular relevance for structural biology and therapeutic development. By reducing the time and resources needed to establish *in vivo* models, this approach has the potential to significantly accelerate research in prion diseases.



Scientific Posters

Prion diseases in animals

Biological Treasures: Biobanks in Service of Prion Science

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Biobanks are organized collections of biological materials and associated data, which have become crucial for advancing scientific knowledge, as well as for diagnosis, research, and experimentation. The Italian Reference Laboratory of Trasmissible Spongiform Encephalopathies (CEA) of the Istituto Zooprofilattico Sperimentale Piemonte, Liguria, and Valle D'Aosta contributes to the Italian veterinary biobank network, managed by the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, and participates in the "WOAH Virtual Biobank" project to create an online catalogue of biological resources. Formalin-fixed and frozen nervous tissues from animals affected by Bovine Spongiform Encephalopathy (BSE), atypical Bovine Spongiform Encephalopathy (L-BSE or BASE), classical and atypical scrapie are stored in the CEA biobank. The collection includes 112 samples of classical BSE, 5 samples of L-BSE, 71 samples of classical scrapie, and 7 samples of atypical scrapie. Tissue selection was performed after microscopic evaluation of slides, based on the presence of target nuclei and the good preservation quality of the tissue. Histological sections were evaluated for neuropil spongiosis, its relative intensity, and neuronal vacuolization within the target nuclei. Immunohistochemical sections were examined to assess the intensity of pathological prion protein (PrP^{Sc}) deposits. For frozen tissue samples, selection was based on tissue quality and the signal intensity of PrP^{Sc} by Western Blot. All scrapie samples were sequenced, allowing for the collection of a variety of genotypes. Biobanks play a crucial role in providing the national and international scientific community with high-quality biological materials for epidemiological, diagnostic, and preventive research, while ensuring its long-term preservation.

Tracing Prions in the Female Reproductive System: Oocytes and Ovaries in Scrapie-Affected Sheep

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Classical scrapie is a prion disease that affects sheep and goats and is primarily transmitted horizontally. However, infection can also occur vertically, from mother to offspring, but the exact mechanism is unknown. Possible routes include infection during pregnancy, birth, or after birth. Prions have been found in reproductive tissues and fluids, suggesting germline transmission. However, no studies have examined prions in oocytes from naturally infected ewes, leaving their role in vertical transmission uncertain. Therefore, the aim of this study is to investigate the presence of prions in oocytes and ovaries of sheep naturally infected with classical scrapie using the ultrasensitive technique PMCA. Histopathological and immunohistochemical evaluations of ovarian tissues have also been performed.

Aditionally, given that multiple studies indicate that genotype and the prion strain can affect prion accumulation in peripheral tissues, including the reproductive system, this study also seeks to assess potential differences in prion accumulation in ovaries and oocytes of sheep of two genotypes (ARQ/ARQ and VRQ/VRQ) and from two distinct scrapie outbreaks.

Our results demonstrate that PrPSc can accumulate in the oocytes and ovaries of scrapie-infected ewes, as detected by PMCA, implicating a potential germline route of transmission. Moreover, this prion accumulation appears influenced by host genotype and prion strain. However, further studies are required to analyze these factors in experimental conditions, with characterized prion strains and known incubation periods to elucidate the mechanisms and implications for disease control and breeding programs.

Atypical BSE cases reported in the absence of BSE-C circulation in Spain from 2015 to 2024

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The classical Bovine Spongiform Encephalopathy (BSE-C) prion agent was responsible for a worldwide food safety crisis in the 1980s. With the majority of cases being detected in the EU, more than 185.000 bovines were affected just in the United Kingdom. In the 1990s, a new form of human prion disease, variant Creutzfeldt-Jakob disease (vCJD), emerged and was later linked to the consumption of BSE-C contaminated products. Measures implemented by the EU authorities included active surveillance programs in Member Countries that allowed the detection of two new variants of BSE, referred to as atypical BSE-H and atypical BSE-L. While BSE-C has an infectious nature, atypical BSE forms have a sporadic origin, being triggered mainly by aging. BSE-C is the only zoonotic prion recognized to date.

BSE-C cases have greatly declined thanks to the measures implemented by the EU authorities. Spain reported its last BSE-C case in 2014 and obtained BSE negligible status from WOAH and EU authorities in 2016. Despite this reduction, atypical BSE cases have been continuously reported in a trickle manner. This work presents and characterizes a total of 12 atypical BSE cases that have been reported in Spain from 2015 to 2024, in the total absence of BSE-C circulating in the country for that period.

Strain characterization of moose Chronic Wasting Disease and its transmission barrier to caribou”

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Chronic Wasting Disease (CWD) is a highly contagious prion disease which already affects deer, elk, and moose in North America and threatens endangered woodland caribou (*Rangifer tarandus caribou*) in Canada. Caribou habitats in the boreal forests overlap with CWD-infected white-tailed deer and moose, where the latter has had an increase in positive cases in the last 3 years. Although the prion protein gene (*Prnp*) is highly conserved in the *Cervidae* family, several polymorphisms that impact CWD propagation and susceptibility have been identified, such as the S138N polymorphism in caribou, which has reduced susceptibility against North American strains. There is a lack of knowledge about *Prnp* polymorphisms, particularly those in Canadian moose, which may contribute to CWD strain variability and potentially infect caribou carrying the protective 138N *Prnp* allele. We have genotyped the *Prnp* gene of moose and found a previously described 209I polymorphic allele in 63.29% of the samples. Among these, 24.05% were homozygous (209II), and 39.24% were heterozygous (M209I). CWD isolates of moose homo- or heterozygous at this codon were characterized by RT-QuIC and inoculated into transgenic mice overexpressing deer PrP, and into our gene-targeted mouse models expressing *Prnp* wildtype, 138SN or 138NN caribou genotypes. Initial findings indicate differences in survival times depending on genotype of the inoculum. Biochemical characterisation and PMCA are currently used to further explore strain differences and transmission barriers. The results of this study are highly relevant for estimating the risk of CWD to our local cervid populations.

Preventing future TSE outbreaks: exploring *prnp* diversity for resistance in Portuguese Goats- setting up a project

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Goats are affected by Classical scrapie (CSc), Atypical scrapie (ASc) and Bovine Spongiform Encephalopathy (BSE), neurological disorders belonging to the Transmissible Spongiform Encephalopathies (TSE). These diseases are characterized by the accumulation of an abnormal protein – PrP^{sc}, an isoform of the cellular prion protein -PrP^{c}. Genetic factors influencing susceptibility to CSc in sheep have been extensively studied, and genetic selection for resistance in sheep is the most effective strategy to control CSc. Studies in different goat breeds detected *prnp* alleles associated with increased resistance to CSc, suggesting that genetic resistance could be an effective tool also in goats. This approach would enable selective culling when managing outbreaks and, at the population level, enhance resilience. However, breeding for resistance must consider the existing allele frequencies in each country to prevent loss of genetic diversity. Data on goat *prnp* alleles conferring resistance to CSc, and especially to ASc, remain limited. In particular, no baseline study on *prnp* variability in goats has been done in Portugal to date. Addressing this gap is particularly important following the recent identification of the first case of CSc in a goat in Portugal.

Our project aims to determine *prnp* polymorphisms in autochthonous and exotic goat breeds raised in Portugal, as well as in a random sample from the national TSE surveillance program, including confirmed TSE-positive goat cases.

A better understanding of *prnp* variability in goats could yield new insights and inform breeding-for-resistance strategies in goats to complement existing disease control measures.

Conservation of strain properties of bank vole-adapted chronic wasting disease in the absence of glycosylation and membrane anchoring

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Prion disease phenotypes (prion strains) are primarily determined by the specific misfolded conformation of the cellular prion protein (PrP^C). However, post-translational modifications, including glycosylphosphatidylinositol (GPI) membrane anchoring and glycosylation, may influence strain characteristics. We investigated whether these modifications are essential for maintaining the unique properties of bank vole-adapted Chronic Wasting Disease (CWD-vole), the fastest known prion strain. Using a novel transgenic mouse model expressing I109 bank vole PrP^C lacking the GPI anchor and largely devoid of glycans, we performed serial passages of CWD-vole prions. Despite elongated initial incubation periods, the strain maintained 100% attack rate through three passages. Although the pathological phenotype exhibited characteristic GPI-less features, including abundant extracellular plaque formation, three subsequent serial passages in fully glycosylated and GPI-anchored bank vole I109 PrP^C expressing transgenic mice TgVole (1x) demonstrated that the strain's distinctive rapid propagation properties were preserved. These findings suggest that neither GPI anchoring nor glycosylation are essential for maintaining CWD-vole strain properties, supporting the concept that strain characteristics are primarily encoded in the protein's misfolded structure.

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Prion detection in the muscles of Norwegian cervids affected by different CWD strains

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Chronic wasting disease (CWD) represents an emerging prion disease within the Nordic countries, having been identified in reindeer, moose, and red deer since 2016. Notably, instances of CWD in moose and red deer across three Nordic countries exhibited distinct pathological and strain characteristics when compared to CWD in reindeer and CWD from North America. This includes an unexpected absence of prions outside the central nervous system, as determined by standard diagnostic methodologies (ELISA, WB and IHC).

However, when exanimated using the protein misfolding cyclic amplification method (PMCA), prions were detected in the lymphoreticular system of both moose and red deer affected by CWD in Norway. Remarkably, prions were also identified in the muscle tissues of these species, as well as in CWD-infected reindeer.

Furthermore, one lymph node and one muscle sample from a moose demonstrated infectivity upon experimental transmission to bank voles.

These findings underscore the systemic nature of CWD strains present in Europe and raise concerns regarding the potential risk of human exposure through the consumption of infected edible tissues.

Improving CWD surveillance in Italy: comparison of diagnostic methods

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CWD surveillance and diagnosis has become an important issue in Europe, since its detection in 2016. Although the diagnostic methods for the active surveillance in bovine and small ruminants were able to detect the European strains, we performed a retrospective study on Italian deer samples collected in the frame of surveillance established by Italian Ministry of Health to compare the results obtained from the authorized test and RT-QuIC method, well-known for its extremely high sensitivity. This is an in vitro amplification method based on the conversion of recombinant PrP substrates in the presence of PrP^{Sc}, defined as "seeding activity". One hundred brainstems and medial retropharyngeal lymph nodes from Italian deers were selected for testing by the HerdChek BSE-Scrapie Antigen Test (IDEXX) and by RT-QuIC. All the samples resulted negative at the rapid test and no seeding activity was detected in any case included in this study, except in one animal only at the level of the brainstem. The brainstem from this case was then submitted to confirmatory TSE analyses, that gave negative results. Although classical confirmatory methods are the official tests to diagnose a case of CWD, given the high sensitivity of RT-QuIC, it is highly probable that this is a pre-clinical case therefore with low PrP^{Sc} presence that classical methods are not always able to detect. Considering that the seeding activity detected by the RT-QuIC method is not always predictive of infectivity only the bioassay analysis will be able to confirm the true pathological status of this animal.

Chronic Wasting Disease (CWD): updates on surveillance of the Italian cervid population

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CWD is a prion disease highly contagious. It is widespread in numerous American States and unfortunately, since 2016, it was identified in some Northern European countries, such as Norway, Finland and Sweden. To monitor its presence, the EU established a surveillance system of the cervid population in six countries. Italy, although not subject to this measure, decided to apply this surveillance system based on specific guidelines issued by the Ministry of Health. According to the actions taken by the EU following the epidemiological data published by the EFSA, since 2023 the surveillance in Italy is focused on *Cervus elephus* species.

In the framework of this surveillance, the targets sampled for the diagnosis were: brainstem (obex) and retropharyngeal lymph nodes. Sampling was entrusted to resident veterinary authorities and the rapid testing to the National Reference Laboratory for TSEs through the Italian Institutes in charge of the active surveillance system for animal prion diseases. These samples were divided into two parts, one of which was fixed in formalin and the other frozen.

Frozen sections of brainstem and retropharyngeal lymph nodes were analysed by the HerdCheck BSE-Scrapie Antigen EIA (IDEXX) and all samples resulted suspect at the rapid test were subjected to the confirmatory analyses. Since 2016 to date more than 3000 samples were analysed and no CWD cases were identified, even if in the framework of a research project, the application of a highly sensitive *in vitro* amplification method such as Real-Time Quaking-Induced Conversion (RT-QuIC) revealed new important diagnostic evidence.

How improvement in BSE and CWD Elisa permitted to deal with complex population

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Prion diagnosis in ruminants became easily widespread 20 years ago mainly by using ELISA for testing BSE, Scrapie and CWD. However, developments are constantly necessary to adapt to the realities in the field. Recently, greater dispersion of negative population values was observed on 1) fresh sheep brains using BSE-scrapie test and 2) specific cervid lymph node samples in the US when tested with CWD kit.

Improvements have been made to address these effects.

1) Regarding the IDEXX BSE-Scrapie test used on fresh sheep brains, the new formulation eliminated the background effect previously observed on a complex sample set. Thus, on 63 Freshly harvested negative samples, the OD mean decreased from 0.129±0.060 to 0.043±0.011, making all samples flat negative. The same observation was made on a set of 23 brains from the EU, with the OD mean decreasing from 0.081±0.073 to 0.041±0.012. In parallel, a positive impact was also observed on 28 fresh bovine brain samples tested in Germany (0.022±0.005 to 0.018±0.002).

2) Concerning the CWD kit and specific reactor samples in the US (n=14), a new formulation of the kit also improved specificity with the OD mean decreasing from 0.278±0.242 to 0.081±0.051 on this complex sample set).

For both kits, sensitivity remained unchanged, reaching 100% for BSE-Scrapie kit and 99.3% on CWD kit. In parallel, the CWD kit also obtained USDA approval for the addition of an Elk claim in April 2025.

This study shows the constant need to monitor field results and to react accordingly by enhancing the diagnostic tools when possible.

Strain Diversity and Cross-Species Transmission Potential of Chronic Wasting Disease Prions to Livestock-Species

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Chronic wasting disease (CWD) is a prion disease affecting cervids, caused by apparently distinct prion strains identified in multiple species across North America and Europe. The transmission abilities of these strains may be affected by amino acid variations in the host PrP sequence.

In this study, we evaluated the interspecies transmission of CWD, focusing on theis ability to infect livestock species and characterizing strain features within distinct livestock-PrP contexts. For that, a panel of CWD isolates derived from different cervid species has been inoculated in transgenic mice expressing PrP from either bovine (Bo-Tg110) or ovine (OV_{VRQ}-Tg338 / OV_{ARQ}-Tg501) species. These isolates have also been used for the study of their in vitro propagation capacity by PMCA.

Our results reveal strain-dependent differences in both the transmission efficiency of CWD isolates to various livestock-PrP transgenic mouse models and their *in vitro* amplification in these PrP contexts.

In the bovine-PrP context, the panel of isolates exhibited at least four distinguishable CWD strains, based on transmission efficiency, attack rates, survival times, PrP^{res} biochemical properties, and *in vitro* amplification capacity. Similarly, in the ovine-PrP context, the isolates displayed at least three distinct CWD strains using the same characterization criteria.

Collectively, these findings underscore the substantial strain diversity present within the panel of CWD isolates and demonstrate that certain CWD agents can cross the species barrier to infect key livestock species. Given the agricultural relevance and potential zoonotic implications, these results highlight the need for further in-depth investigation into the transmission dynamics and risks associated with CWD.

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Diagnostics and epidemiological investigations of emerging animal prion diseases in Tunisia

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The recent identification of classical scrapie (CS) in sheep in Tunisia and in Libya, as well as of a new prion disease affecting dromedary camels (CPrD) in Algeria and Tunisia, has raised significant concerns about the distribution and potential spread of these diseases in North Africa.

Within the framework of the "Italy-Africa Research Doctorates" program (promoted by the Istituto Superiore di Sanità and the University La Sapienza), a research project has been launched to investigate the transmission dynamics and ecological impact of animal prion diseases in Tunisia.

The study is a pilot focusing on the epidemiological characterization of CS and CPrD in small ruminants (SR) and dromedary camel populations, in order to support the development of future surveillance. This includes general demographic analysis of SR and dromedary camels in Tunisia, case detection, biochemical and histopathological characterization, determination of PrP genotype and the investigation of prion distribution in dromedaries affected by CPrD.

Preliminary data were collected through an initial field investigation targeting SR and dromedary camel populations, with a particular focus on putative risk factors linked to farming practices and environmental exposure.

Ongoing work includes active sampling of high-risk groups. Collected specimens are being analysed through genetic, neuropathological and biochemical methods, including molecular PrP^{Sc} typing.

This study aims to generate new scientific data on the prevalence, pathological features, and risk factors associated with CS and CPrD in Tunisia. The findings will support the establishment of targeted surveillance strategies and inform public and animal health policies at both national and regional levels.

Thermal Inactivation of Prions in Animal By-Products: Efficacy Assessment of Autoclave-Based Decontamination Treatments

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Transmissible spongiform encephalopathies (TSEs), such as scrapie in sheep and goats, are fatal neurodegenerative disorders caused by prions—infectious protein agents known for their self-replicative properties and high resistance to degradation. The persistent nature of prions complicates the safe reuse of animal by-products not intended for human consumption (SANDACH), which possess significant economic potential.

The present study aimed to evaluate the effectiveness of various thermal decontamination treatments using subcritical hydrolisis under different time and temperature conditions to enable the safe reuse of these materials for the extraction of peptones. Homogenates of scrapie-infected ovine and transgenic murine (Tg338) brains were subjected to treatments ranging from 120 to 140°C for periods of 1 to 4 hours. The prion content post-treatment was assessed using Protein Misfolding Cyclic Amplification (PMCA), combined with Dot Blot, Western Blot, and bioassay in Tg338 mice.

Results indicated that treatments at 120 °C were insufficient, while temperatures of 130–133 °C for extended durations significantly reduced or nearly eliminated murine prions, although ovine prions required more stringent conditions. The most effective protocols were then applied to contaminated and uncontaminated meat and bone meal (MBM) types III and I, respectively, confirming their efficacy. These findings suggest that specific thermal protocols can achieve prion inactivation, potentially allowing safe reutilization of SANDACH. However, further research is necessary to assess the impact of these treatments on the chemical integrity of the treated materials.

Development of ovine-based *in vitro* models for prion diseases

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The study of prion diseases still relies heavily on *in vivo* experimentation, which is not only time-consuming and costly, but also ethically controversial. As an alternative, we propose an *in vitro* model based on the ovine species, which plays a key role in prion research.

This model is based on the generation of neurospheres using brain cells from adult sheep. Neurospheres are 3D structures composed of multiple cell types capable of differentiating into various central nervous system lineages. For this purpose, adult neural stem cells were extracted from the subventricular zone with the aim of generating neurospheres. Cells were seeded at 100,000 cells/mL and cultured for 30 days in medium enriched with growth factors to prevent premature differentiation. As neurospheres reached 100 µm in diameter, cultures underwent trypsinization to promote cell dissociation and increase neurosphere yield and uniformity. This process was repeated until a homogeneous population of similarly sized, debris-free spheres was achieved.

This study demonstrates that it is feasible to obtain neurospheres from the adult ovine brain, enabling the generation of numerous *in vitro* experimental units from a single animal. This represents a promising step towards reducing animal use while maintaining species specificity. Moreover, neurospheres could serve as a foundation for creating more complex 3D models, such as brain organoids.

Evidence of a single vole-adapted strain from North American CWD isolates in vole carrying methionine at PrP residue 109

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Chronic wasting disease (CWD) is a prion disease affecting cervids that is spreading in the US and Canada (CA). Experimental studies suggest that a mixture of strains may circulate in CWD-affected cervids in North America. Investigation of strain variability is critical for assessing zoonotic risk and monitoring the spread of CWD. In previous studies, we reported the isolation of a single vole-adapted strain from US- and CA-CWD isolates in bank voles carrying isoleucine at PrP residue 109 (BvI). In the present study, we extended our research by performing strain typing in voles carrying methionine (BvM) at PrP residue 109, as an alternative vole model. In particular, we compared strain properties of eleven isolates from elk, moose, mule deer, and white-tailed deer collected in CA and the US. All CWD isolates were transmitted in BvM, although with a longer incubation time than we previously reported in BvI for the same isolates. Second passages of seven of these isolates have been completed, showing similar clinico-pathological phenotypes. The isolation of a single BvM-adapted strain so far, regardless of the geographical origin or source species, may reflect the selective propagation of a strain component common to all isolates. Importantly, our data will be useful for comparing strain properties of North American CWD isolates with those of prion-affected cervids recently found in Northern Europe.

Spatial patterns of classical scrapie in small ruminants and Creutzfeldt-Jakob disease in humans: a geographical approach to investigate the zoonotic potential of scrapie.

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Abstract:

Classical scrapie (CS) is a transmissible spongiform encephalopathy with a hypothesized, though unconfirmed, zoonotic potential. This study investigated that potential by comparing the geographical distribution of CS in Italy with that of Creutzfeldt-Jakob disease (CJD). Accounting for a latency period, data from 135 CS outbreaks identified through testing 31,552 herds (01/01/2002 - 31/12/2006) and 506 CJD deaths (01/01/2010 – 31/12/2014) were analysed. Standardized mortality ratios (SMRs) were calculated at provincial level, adjusted for flock-level surveillance intensity (CS), age and sex (CJD). A latency period of 4 to 12 years was considered as a plausible latency period for CJD after exposure to CS. Geographical patterns were assessed using Bayesian Poisson-Gamma and Besag-York and Mollié (BYM) models, separately for each disease. Bayesian shared components models were used to identify areas with common spatial risk. Finally, an ecological regression model was used to assess the association between CJD (outcome) and CS (exposure). Both Poisson-Gamma and BYM models revealed spatial heterogeneity for CS and CJD, highlighting high-risk areas in Sicily, Sardinia and central Italy. The Bayesian shared component model suggested the presence of areas with latent risk factors common to both diseases. The ecological regression model indicated a weak but positive association between the distribution of CS and CJD (β=0.02; 95% CrI: -0.0105 -0.05581). In conclusion, the consistency of findings across multiple Bayesian models supports the hypothesis of overlapping spatial risk factors for CS and CJD. Although the ecological association was weak, it is consistent with the hypothesis of the zoonotic potential of scrapie.

Classical Scrapie in Icelandic Sheep – A Risk Factor Analysis

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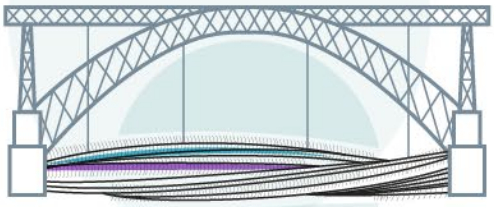
Classical Scrapie is a fatal neurodegenerative disease affecting sheep, with a huge impact on economic sheep farming and animal welfare. Iceland has implemented extensive control measures to eradicate the disease, but in certain regions outbreaks continue to occur. Therefore, this study aims to identify potential risk factors for disease transmission based on survey data from affected and non-affected farms across different regions of Iceland. For this a questionnaire survey was conducted in affected and non-affected farms in different regions of Iceland collecting data on farm characteristics (land & flock size), farm management, animal movements, personal contacts and biosecurity measures.

A total of 49 sheep farmers were surveyed, including all sheep farmers who had been diagnosed with scrapie in the last 10 years. Based on this questionnaire, potential risk factors were identified, and regional differences were analysed. The main risk factors for the persistence of Classical Scrapie in Iceland are shared pastures, birth hygiene as well as human contacts and shared machinery. Communal grazing may increase the risk. Spreading infected manure on pastureland can lead to a long-term risk of reinfection with scrapie. Burying dead animals on the farm could also permanently contaminate the soil. Animal movement and multiple human contacts further influence disease spread, i.e. larger farms, with more animals and higher interaction, face greater risks. To reduce future outbreaks, strengthening biosecurity, breeding for genetic resistance, and improving carcass disposal are key strategies for eradication of Scrapie in Iceland.



Scientific Posters

Prion Diseases and Prion-Like diseases in humans

Accurate Detection of Pathologic α-Synuclein in CSF, Skin, Olfactory Mucosa, and Urine with a Uniform Seeding Amplification Assay

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Currently, early diagnosis of dementia with Lewy bodies (DLB) is based on clinical criteria, which is challenging due to overlapping symptoms with other neurodegenerative diseases. Seeding amplification assays are emerging as a promising diagnostic tool for detecting pathological α-synuclein (αSyn^D) including DLB and Parkinson's disease. This study aimed to test whether the same seeding amplification assay established for αSyn^D detection in cerebrospinal fluid (CSF) could be applied to skin, olfactory mucosa, saliva, and urine, obtained from the same patients. A total of 31 patients with probable DLB and 53 healthy controls were recruited. When evaluating the assays' applicability to different biospecimens, only those collected from participants with a positive CSF αSyn^D result were considered. Seeding amplification assay results were evaluated based on the αSyn^D amplification rate over 48 hours and the value of the area under the curve. The sensitivity and specificity were 94% and 98% for skin, 47% and 100% for olfactory mucosa, and 22% and 100% for urine, respectively for the CSF positive DLB and healthy controls. αSyn^D was undetectable in saliva. Cohen's Kappa analysis (κ) showed almost perfect agreement between CSF and skin assays (κ=0.86) but slight to no agreement for CSF versus olfactory mucosa (κ=0.12) and urine (κ=0.094). In summary, the seeding amplification assay established for αSyn^D detection in CSF demonstrated comparable diagnostic performance in minimally invasive skin biopsies. Olfactory mucosa, saliva, and urine sample preparation pose technical challenges resulting in the established assays' low diagnostic accuracy, for now, but potential for non-invasive diagnostics of α-synucleinopathies.

A novel structure-driven strategy for Parkinson's disease immunotherapy

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Parkinson's Disease (PD) is the second most common and fastest-growing neurodegenerative disorder, yet no treatments can halt or reverse its progression; current therapies can help manage early symptoms, but they do not slow or stop disease progression.

The characteristic loss of dopaminergic neurons in the substantia nigra leads to motor symptoms like bradykinesia, tremors or rigidity. The principal pathological hallmark of PD is the accumulation of insoluble protein inclusions in the brain, named Lewy bodies, primarily composed of aggregated α-Synuclein (αSyn). During the amyloid aggregation process of αSyn a prefibrillar toxic intermediate is formed, the oligomer. αSyn oligomers are considered to be the most toxic species in PD accounting for in-brain propagation of the disease and homeostasis disruption. Our team has worked to identify a structurally defined region only present in oligomeric species that mediates the oligomer-to-fibril conversion.

Nanobodies are small, single-domain antibody capable of binding antigens with high precision. Due to their small size and high stability, they are suitable for a wide range of therapeutic applications, diagnostic procedures and research initiatives.

Here, we propose a new potential immunotherapeutic approach that combines structurally informed rational design with cutting-edge AI technology to create a series of nanobodies that target this specific region orchestrating the oligomer-to-fibril conversion over any other species.

Our preliminary results suggest that these nanobodies can impact αSyn aggregation kinetics and that selective binding can be achieved to the oligomeric species, thus supporting the potential of this new approach.

Pedigree Analysis and Genetic Inheritance of Fatal Familial Insomnia (FFI) in a Portuguese Multigenerational Family

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Fatal familial insomnia (FFI) is a rare, autosomal dominant prion disease caused by a mutation in the PRNP gene, leading to the misfolding of the cellular prion protein (PrPC) into its pathogenic form (PrPSc). This results in neurodegeneration, particularly in the thalamus, a key region regulating sleep-wake cycles, which underlies the hallmark symptoms of FFI, including insomnia, autonomic dysfunctions, motor disturbances and cognitive decline. This study focuses on a Portuguese family with FFI, providing a detailed pedigree analysis spanning five generations and comprising 125 individuals, to elucidate inheritance patterns, disease onset, and clinical progression.

The findings confirm the autosomal dominant inheritance pattern and a strong familial clustering of the disease with age of onset in the late 50s (mean 57 years). While 67% of affected individuals succumbing to the disease within months to 1.5 years, a notably 33% exhibited prolonged survival beyond the typical disease duration, exceeding proportions reported in the literature.

Prodromal symptoms, including generalized pain, headaches, tinnitus, pruritus, and behavioral changes, were noted to appears up to five years before diagnosis. In some cases, disease onset was associated with psychological stressors, such as emotional stress and mourning, providing insights into early disease manifestations.

The identification of prodromal symptoms and variability in disease onset and duration reinforces the need for early recognition and surveillance in at-risk individuals. Further research integrating genomic sequencing, biomarkers, and longitudinal clinical assessments are needed to better understand the mechanisms underlying the heterogeneity of FFI and to explore potential therapeutic interventions.

Combined Morphology and Protein Profiling Grades Microglial Reactivity across Neurodegenerative Proteinopathies and Brain Regions

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Introduction

Transcriptomic studies have highlighted significant diversity of microglia and their reactivity to misfolded proteins across different neurodegenerative diseases and brain regions.

Objectives

Investigate such microglial differences on protein and morphology level when comparing Alzheimer's disease (AD) and Creutzfeldt-Jacob's disease (CJD) using novel targeted proteomics together with digital morphological profiling.

Materials and methods

Post-mortem human FFPE brain tissue from both the frontal and occipital neocortex of AD (n=5), CJD (n=5) and control (n=2) patients were processed in Nanostring's GeoMx Digital Spatial Profiler platform to obtain immunofluorescence images of IBA1-stained cells. Targeted antibody-based proteomics was performed on isolated IBA1+ segments in the platform, while digital morphological profiling was conducted on segmented IBA1+ objects through CellProfiler. Combined quantitative reactivity grading was established using both protein and morphology data with trajectory analysis in R.

Results

Morphological profiling of IBA1+ objects distinguished a ramified subtype and two ameboid subtypes. The ramified subtype was the most common subtype in controls and was associated with homeostatic microglia proteins like TMEM119 and P2ry12. One of the ameboid subtypes was linked to inflammatory markers, like HLA-DR and CD11c, and was more prevalent in CJD patients. Reactivity grading could order the patient groups with lowest scores for controls and highest scores for CJD. Most interestingly, occipital cortex samples within both AD and CJD patients had higher reactivity scores than those from the frontal cortex.

Conclusions

Both protein expression and morphometrics of IBA1+ cells can be synergistically joined to grade the reactivity of microglia across different proteinopathies and brain regions.

National Biobank for Genetic CJD: A Novel Collaborative Platform Led by Patient Advocacy, Scientists, Physicians, and Regulators.

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The E200K mutation-related genetic Creutzfeldt-Jakob Disease (gCJD) has an unusually high incidence among the Libyan-Tunisian Jewish population in Israel. In response, the Creutzfeldt-Jakob Foundation Israel, in collaboration with Soroka University Medical Center and the Midgam National Biobank, launched the first national biobank dedicated to gCJD in 2021. The biobank serves as a knowledge-sharing platform and research infrastructure to promote scientific collaboration, early diagnosis, biomarker discovery, and the development of future treatments.

To date, the biobank has enrolled 304 individuals: 25 symptomatic genetic carriers and 279 healthy first- and second-degree relatives, aged 15 to 90. Among them, 86 individuals have provided repeated samples. Biospecimens include plasma, serum, PBMCs, whole blood, urine, saliva, and nasal swabs. Clinical data are extracted from electronic health records, and the dataset is enriched by self-reported lifestyle and psychological information.

This biobank represents a unique opportunity to accelerate research into this rare disease by centralizing biological and clinical data and ensuring open access to research outcomes. The platform also integrates AI-based analysis. The biobank's model and achievements were recently published in the Orphanet Journal of Rare Diseases (Anane et al., 2025), highlighting its global potential in advancing the understanding of gCJD pathophysiology and identifying therapeutic targets.

Genetic Spectrum of Human Transmissible Spongiform Encephalopathies in Spain: Epidemiological Trends and Novel *PRNP* Variants

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Genetic forms of human transmissible spongiform encephalopathies (HTSEs) constitute a clinically and genetically heterogeneous subgroup of prion diseases. As of March 2025, the Spanish National Registry (RNEETH), coordinated by the National Centre for Epidemiology at Instituto de Salud Carlos III (ISCIII), has recorded 202 genetic HTSE cases, representing 7.7% of national notifications. The most frequent mutations were E200K (45%) and D178N-M129 (40%), associated with familial Creutzfeldt-Jakob disease (fCJD) and fatal familial insomnia (FFI), respectively, with distinct geographic patterns across autonomous regions. Less frequent variants included D178N-V129 (5.4%), typically linked to fCJD P102L (1.5%), and Y218N (2.0%), associated with Gerstmann-Sträussler-Scheinker syndrome (GSS).

In addition to registered cases, our laboratory has identified 12 PRNP variants not yet reported to the Registry. These include five rare (G54S, R136S, R148H, N171S, R208H) and six novel variants (G62S, H96Y, V161A, G195R, M205I, V209E), most of uncertain clinical significance. Structural and evolutionary analyses suggest that certain novel variants (V161A, G195R, V209E) are non-conservative substitutions affecting highly evolutionarily constrained regions of the prion protein, potentially altering its stability. In contrast, other changes, particularly in the flexible N-terminal region (G62S, H96Y) or involving conservative substitutions (M205I), may have limited structural or functional consequences.

These findings underscore the diagnostic challenges of classifying rare or novel PRNP variants, especially in the absence of family history or supporting literature. Systematic notification of all identified variants is essential to strengthen genetic counseling, enhance epidemiological surveillance, and support international efforts in prion disease research and policy.

Declining Diagnostic Efforts in Human Prion Disease Surveillance in Spain (1993–2025): An Urgent Call for Action

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Human transmissible spongiform encephalopathies (HTSEs) are rare, fatal neurodegenerative disorders that are subject to mandatory notification. In Spain, the National Registry of HTSEs has systematically collected data since 1993. As of March 31, 2025, a total of 2618 cases have been reported: the majority correspond to sporadic Creutzfeldt-Jakob disease (sCJD, 76.9%), followed by non-CJD cases (14.4%), familial CJD (4.2%), fatal familial insomnia (3.2%), iatrogenic CJD (0.3%), variant CJD (0.2%), and Gerstmann-Sträussler-Scheinker syndrome (0.4%).

Although the Spanish surveillance system remains solid, a marked decline in case notifications and the use of ancillary diagnostic tests has been observed since 2020. Ancillary diagnostic tests, such as brain magnetic resonance imaging, electroencephalography, cerebrospinal fluid 14-3-3 protein detection, genetic analyses, and, more recently, real-time quaking-induced conversion (RT-QuIC), are essential for accurate case classification. However, 14-3-3 protein and genetic testing have notably decreased in recent years. RT-QuIC, introduced in 2018 to enhance diagnostic sensitivity, has not compensated for this decline and was performed in only 31% of cases in 2024.

This reduced implementation of biochemical and genetic tools may impair diagnostic precision and compromise the international comparability of surveillance data.

It is therefore crucial to reinforce the timely and complete notification of suspected HTSE cases and ensure the consistent application of recommended diagnostic protocols in accordance with updated EuroCJD surveillance criteria. Maintaining a high standard of epidemiological surveillance is essential for tracking trends, improving diagnostic accuracy, and aligning with European and global health monitoring efforts.

Exploring the presence of soluble amyloid beta aggregates in young non-AD patients.

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Recently, pre-amyloid Aβ seeds have been detected in the brain of young, pre-depositing Tg mice, suggesting that such pathogenic seeds might also be present in AD patients before the start of Aβ pathology (early seeds). Those seeds conformation and/or biochemical nature might be different from Aβ seeds isolated from brains of late-stage AD patients, therefore being a potential therapeutic target to stop Aβ pathology in its earliest phase. Here, using biochemical and microscopy techniques we explore the existence of Aβ seeds in young (<65 y.o.) non-AD patients. Combining ultracentrifugation, NaPTA precipitation, DEA extraction and ELISA, we detected Aβ signal in samples from non-AD patients, suggesting the presence of soluble Aβ aggregates. However, such results couldn't be confirmed by oligomeric ELISA. On the other hand, while immunogold labelling and electron microscopy seems to be a promising tool to detect seeds in complex samples, western blotting results are inconsistent, not allowing any conclusions to be drawn. Although the results obtained in no case confirm the existence of Aβ seeds in non-AD patients, they do offer some clues on how to assess for the potential presence of such seeds.

PMCA optimization for the detection of prions in blood and urine of patients with Fatal Familial Insomnia

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Fatal Familial Insomnia (FFI) is a rare prion disease caused by a mutation in the *PRNP* gene, resulting in a substitution of asparagine with aspartic acid (D178N). Genetic testing can identify mutation carriers, but biomarkers capable of predicting disease onset are still lacking. This study aims at developing a new protein misfolding cyclic amplification (PMCA) protocol to detect prions (PrP^{Sc}) in urine and blood samples of FFI mutation carriers collected at different disease stages (preclinical and clinical). To this aim, urine samples longitudinally collected from D178N mutation carriers at preclinical (n=fourteen) or clinical (n=four) stage and controls (n=twenty) were tested, as well as one plasma sample collected from a patient at terminal disease stage. PMCA enabled the detection of prions in the urine of ten mutation carriers, two symptomatic and eight asymptomatic at the time of collection. The PMCA analysis of the plasma also yielded a positive result. These findings suggest that PMCA may be capable of detecting prions prior to the onset of symptoms; however, it remains unclear whether—and to what extent—this could serve as a reliable biomarker for disease initiation. Further evaluation of the patients' clinical parameters (e.g., kidney functions/dysfunctions) could help clarify why prions were detectable in the urine of some asymptomatic carriers but not others, and provide insights into the reliability and timing of prion detection.

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