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8th IBERIAN CONGRESS ON PRIONS 2019

Abstract Book

24th and 25th October 2019

Instituto Nacional de Investigação Agrária e Veterinária, I.P.

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8th IBERIAN CONGRESS ON PRIONS 2019

PROGRAM

THURSDAY 24th OCTOBER

8:30-10:00	Registration
9:30	Welcome
PRION STRUCTURE AND BIOLOGY	
10:00-11:00	Chair: **Jesus Requena** Invited Speaker: Professor **Markus Glatzel** (Institute of Neuropathology, University Medical Center Hamburg – Eppendorf) **- "The role of proteolytic processing of the prion protein in neurodegenerative diseases"**
11:00-11:30	Coffee Break and Poster Discussion
11:30-13:00	Chairs: **Salvador Ventura** and **Jesus Requena** Oral Communications: 1. **Giovanni Spagnolli** (CIBIO-University of Trento) **- "Unraveling Common Molecular Mechanisms of Prion Propagation"** 2. **Yaiza B. Codeseira** (CiMUS-University of Santiago de Compostela) **- "A Structural Insight of Infectious Recombinant PrP^{Sc} Using FTIR"** 3. **Hasier Eraña** (CIC bioGune, ATLAS Molecular Pharma) **- "A largely scalable new method to produce infectious recombinant prions"** 4. **Susana Navarro** (IBB, UAB) **- "A first bacterial extracellular and functional prion-like protein"** 5. **Yanick Bichot** (Biorad) **- "A complete and consistent solution for TSE diagnosis"**
13:00-14:30	Lunch
14:30-15:30	Chair: **Jesus Requena** Invited Speaker: Professor **Maria João Saraiva** (Molecular Neurobiology group - Instituição de investigação e inovação em saúde i3S and IBMC - University of Porto) **- "Animal models of transthyretin amyloidosis to search for FAP patient´s biomarkers"**
PRION DISEASES IN ANIMALS	
15:30-16:30	Chairs: **Enric Vidal** and **Hasier Eraña** Oral Communications: 6. **Alba Marín-Moreno** (CISA-INIA) **- "Broad study of the diversity of classical scrapie prions circulating in Europe by using just two rodent models"** 7. **Tomás Barrio** (Centro de Encefalopatías y Enfermedades Transmisibles Emergentes, University of Zaragoza) **- "Mixtures of prion substrains in field cases of scrapie revealed by mice bioassay"** 8. **Marina Betancor** (Centro de Encefalopatías y Enfermedades Transmisibles Emergentes, University of Zaragoza) **- "Therapeutic assay with the non-toxic C-terminal fragment of tetanus toxin (TTC) in transgenic murine models of scrapie"**
16:30-17:00	Coffee Break and Poster Discussion
17:00-18:00	Chairs: **Tomas Mayoral** and **Juan Badiola** Oral Communications: 9. **Diego Sola** (Centro de Encefalopatías y Enfermedades Transmisibles Emergentes, University of Zaragoza) **- "Infectivity study: inoculation of tg340 mice with tissues from resistant goats inoculated with bovine and caprine bovine spongiform encephalopathy"** 10. **Mafalda Casanova** (University of Evora, INIAV)" **- "Characterization of the first Portuguese cases of Atypical Scrapie in transgenic ovine ARQ-PrP mice"** 11. **A. Hernaiz** (LAGENGIO, University of Zaragoza) **- "Whole genome DNA methylation profiles in the central nervous system of sheep naturally infected with scrapie"**
18:00	Musical moment by ***Castra Leuca Trio***

8th IBERIAN CONGRESS ON PRIONS 2019

PROGRAM

FRIDAY 25th OCTOBER

PRION DISEASES IN ANIMALS (continuation)	
9:00-10:00	Chair: **Leonor Orge** Invited Speaker: Doctor **Sylvie Benestad** (Norwegian Veterinary Institute, Oslo) **- "CWD Norwegian experience, diagnosis and control, risk analysis and characteristics of the Norwegian CWD cases"**
10:00-11:00	Chairs: **Rosa Bolea** and **M. Lurdes Pinto** Oral Communications: 12. **Raymond Bujdoso** (Cambridge University) **- "Susceptibility of human PrP *Drosophila* to cervid prions"** 13. **Christopher J. Silva** (USDA) **- "Quantitating prion polymorphisms from heterozygous CWD-infected white-tailed deer"** 14. **Alicia Otero** (University of Alberta) **- "Elk-PrP^{C} expression levels do not alter the Wisc-1 CWD strain properties and favors its selection from mixtures"**
11:00-11:30	Coffee Break and Poster Discussion
11:30-12:00	15. **Amy L. Robinson** (Roslin Institute) **- "Regional variation in the prion protein gene (*PRNP*) in wild European deer species"** 16. **Jorge C. Pereira** (CITAB, CECAV, UTAD) **- "Chronic wasting disease risk assessment in Portugal - Genetic variability preliminary results and future perspectives"**
PRION DISEASES AND PRION-LIKE DISEASES IN HUMANS	
12:00-13.00	Chair: **Joaquin Castilla** Invited Speaker: Doctor **Elvan Boke** (Centre for Genomic Regulation, Oocyte Biology and Cellular Dormancy group, Barcelona) **- "The Balbiani Body: A super-organelle held together by amyloid-like assembly"**
13:00-14:30	Lunch
14:30-16:30	Chairs: **Juan Maria Torres** and **Joaquin Castilla** **- 9th Iberian Congress on Prions 2020** Oral Communications: 17. **Benoit Schneider** (Université Paris Descartes, Inserm) **- "Three pathological consequences of TACE α-secretase deregulation in prion diseases"** 18. **Franc Llorens** (CIBERNED, IDIBELL, University Medical School, Goettingen) **- "Plasma total prion protein as a potential biomarker for neurodegenerative dementia: diagnostic accuracy in the spectrum of prion diseases"** 19. **Charlotte M. Thomas** (Roslin Institute) **- "Optimising RT-QuIC for the detection of prion seeding activity in BSE-infected ovine blood."** 20. **Juan Carlos Espinosa** (CISA-INIA) **- "Amino acid residues in β2-α2 loop of human-PrP regulate prion strain susceptibility"**
16:30-17:00	Coffee Break and Poster Discussion
17:00-18:00	Chair: **Franc Llorens** Oral Communications: 21. **O. Andreoletti** (UMR INRA ENVT) **- "sCJD agents distribution in peripheral tissues"** 22. **Abigail B. Diack** (Roslin Institute) **- "vCJD strain is consistent in individuals of two *PRNP* codon 129 genotypes"**
20:00	Congress Dinner

Index

Invited Speakers

Professor Doctor **Markus Glatzel** (Professor of Neuropathology and Director of the Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany)

Professor Markus Glatzel graduated and doctorate in Medicine in 1997 at Medical School, University of Ulm and Freiburg, and got his habilitation in Neuropathology in 2005 at University of Zurich. Between 1997 and 2002 Prof. Glatzel was first a Postdoctoral Research Fellow and then a resident in Patology and Neuropathology in the Laboratory of Professor Doctor A. Aguzzi at University of Zurich where he stayed as a consultant for Neuropathology until 2006.

Since then Professor Glatzel is the Director of the Institute of Neuropathology and Professor of Neuropathology, UKE, University of Hamburg, being also Chairman of the Diagnostic Center, UKE, at the same University since 2011. Moreover Professor Glatzel is editor in Chief of "Brain Pathology" journal since 2019.

Professor Glatzel has authored an extensive work articles in peer reviewed journals and participates in various research projects with the aim of identifying the molecular basis of neurodegeneration focusing on protein folding diseases (prion diseases, Alzheimer disease, neuroserpin-dementia) with the following major areas: cellular and molecular mechanisms of disturbed protein degradation in dementia and translational research investigating molecular basis of divergent phenotypes of dementia.

Professor Glatzel was distinguished with the Pfizer Award for Neuroscience (2003), the Georg-Friedrich-Götz-Award (2004) and the Cavanagh Price of the British Neuropathological Society (2007).

Prion Structure and Biology

The role of proteolytic processing of the prion protein in neurodegenerative diseases

Neurodegenerative diseases such as Alzheimer's disease or prion diseases are untreatable debilitating diseases occurring mainly in the elderly.
For these diseases, molecular mechanisms of neurodegeneration are only partially understood. Aberrant proteolytic processing of neuronally expressed proteins leading to disturbed neuronal protein homeostasis represents one mechanism leading to neurodegeneration.
The membrane-anchored cellular prion protein plays detrimental roles in neurodegeneration by its ability to misfold into a pathogenic isoform (in prion diseases) and by acting as a neuronal receptor for toxic protein oligomers (e.g., in Alzheimer's disease). The mature form of the prion protein is processed by a variety of proteolytic cleavage events including shedding by the metalloprotease ADAM10.
Using cellular models and genetically modified mice, we study the impact of proteolytic processing of the cellular form of the prion protein on its physiological functions and its influence on neurodegenerative diseases.
Here, we report our most recent findings on the physiological and pathological relevance of these processing step and present an update on our experimental strategy of exploiting these cleavage events as a therapeutic option in neurodegenerative diseases.

Professor Doctor **Maria João Saraiva** (Principal Researcher of Molecular Neurobiology group - Instituição de investigação e inovação em saúde i3S and IBMC- University of Porto, Portugal)

Maria João Mascarenhas Saraiva received a BSc in Biology from the University of Porto, Portugal, in 1976, and an MSc in Biochemistry from the University of London, in 1978. Between 1980 and 1984, she did a PhD in biochemistry at the University of Porto, and qualified as Professor of Biochemistry in the University of Porto in 1991. She worked for different periods as a Visiting Scientist at the College of Physicians and Surgeons at Columbia University, New York. She is Director of the Molecular Neurobiology Group at i3S and IBMC in Porto University.

Maria J Saraiva was awarded the Seiva Prize for Services to Science by the City of Porto, in 1996, and the Gulbenkian Prize in Science, in 2009. She has published over 230 articles in peer reviewed journals, several reviews on the subject of molecular biology of misfolding diseases of the central and peripheral nervous system.

In 1996 was awarded Seiva Prize for services on Science to the City of Porto, and in 2009 was distinguished with Gulbenkian Prize on Science.

Prion Structure and Biology

Animal models of transthyretin amyloidosis to search for FAP patient´s biomarkers

Novel insights regarding Familial Amyloidotic Polyneuropathy (FAP) pathogenesis and mechanisms underlying nerve degeneration are paramount for the development of novel therapeutic strategies or disease following biomarkers. Microarray-Based Gene Expression Analysis has been used to search for alterations in the transcriptional machinery in peripheral nerve and dorsal root ganglia of a pre-clinical FAP mouse model carrying the TTR V30M mutation, in a heterozygous Hsf-1 background (Hsf/V30M), presenting TTR non-fibrillar deposition in the PNS. Over and down expressed genes relative to non-transgenic controls were then analyzed following a protocol template that encompasses both the pre-clinical model and human clinical samples. Tissues and plasma samples are investigated by RNA and protein to confirm differential expression of the markers found , their location and co-relation with deposition determined. Since treatment of Hsf/V30M mice with Anakinra or TTR siRNA prevents TTR non-fibrillar deposition in the PNS either by decreasing inflammation or silencing liver TTR synthesis, respectively, the above described analyses are repeated upon treatment and compared.
Following ALL these criteria altered expression of extracellular matrix genes was found. Matrix metalloproteases (MMPs) are endopeptidases, identified as matrix-degrading enzymes that regulate fundamental biological process for normal growth, development and repair. Additionally, robust association with AXON GUIDANCE molecules that account for nerve growth cone formation was also strong.
This approach is the basis for a long term project of several signature biomarkers in patients carrying different TTR mutations and in the follow up of current and future therapies for FAP.

Doctor **Sylvie Benestad** (Head of the National Reference Laboratory for TSE in animals-Norwegian Veterinary Institute, Oslo, Norway and Head of the OIE reference laboratory for CWD, Norwegian Veterinary Institute Oslo)

Sylvie Benestad obtained her PhD in Neurophysiology in 1994 in the University of Marseille-Aix (France) collaborating with the University of Oslo (Norway). She is working at the Norwegian Veterinary Institute since end of 1997, working almost exclusively with TSE diagnostic and research specializing in the identification and characterization of animal prion diseases. She is the head of the National Reference Laboratory for TSE in animals. The World Organization for Animal Health (OIE) has designated the Norwegian Veterinary Institute as the third reference laboratory for Chronic Wasting Disease (CWD) in the world, and the first one in Europe. Senior researcher Sylvie Benestad has been designated as expert, responsible for the new reference laboratory.

CWD Norwegian experience, diagnosis and control, risk analysis and characteristics of the Norwegian CWD cases.

Benestad SL (1), Tran L (1), Vuong T (1), Madslien K (1), Pirisinu L (2), Vaccari G (2), Bian J (3), Moreno JA (3), Kim S (3), Telling GC (3) R, Moda F (4), Bistaffa E (4), Diack A (5), Andréoletti O (6), Nonno R (2), Vikøren T (1), Våge J(1)

(1) Norwegian Veterinary Institute, Oslo, Norway, (2) Istituto Superiore di Sanità, Department of Veterinary Public Health, Nutrition and Food Safety, Rome, Italy, (3) Colorado State University, Prion Research Center, Fort Collins, CO USA, (4) Istituto Neurologico Carlo Besta, Milan,Italy, (5) The Roslin Institute, University of Edinburgh, UK, (6) INRA/ EVT Toulouse, France

Chronic wasting disease (CWD) was detected in North America (Colorado) for the first time in 1967 and is now diagnosed in captive and free-ranging cervids (mule deer (Odocoileus hemionus), white-tailed deer (Odocoileus virginianus), elk/wapiti (Cervus Canadensis) and moose (Alces alces)) in 26 American states and 3 Canadian provinces. CWD is still spreading despite considerable efforts to restrain the disease. CWD has also been diagnosed in red deer (Cervus elaphus) and sika deer (Cervus nippon) in South Korea as the result of importing CWD infected elk from North America.

CWD is considered as the most contagious of the prion diseases, transmitted by direct contact from deer to deer, or in some extend from mother to offspring, or indirectly through contact with environment contaminated by feces, saliva, urine or carcass from infected animals.

In April 2016 CWD was diagnosed for the first time in Europe, in a wild reindeer (Rangifer tarandus), in the Nordfjella area in Southern Norway (Benestad et al. 2016). CWD in the Norwegian reindeer showed diagnostic characteristic indistinguishable from those of the CWD cases identified in North America. Norway immediately extended the surveillance program for CWD with substantial sampling in the whole country, based on results from modelling of the disease. Drastic measures for eradication of CWD were implemented between in the Nordfjella reindeer population. Culling of this population was finalized spring 2018, resulting in a total of ca 2424 animals tested. Of these 19 were diagnosed with CWD, indicating a prevalence under 1%.

Over 90 000 cervids are now tested. In total 25 CWD cases have been detected. In addition to the 19 positive reindeer, all from Nordfjella, 5 moose (Alces alces) and one red deer (Cervus elaphus), have been identified as positive for PrPSc. These 6 animals are old (13-20 years) and found in 4 distinct populations approximately 300 to 500 km away from Nordfjella.

Preliminary bioassay results show that both the CWD type of the moose that displayed clear differences in the host (Pirisinu et al. 2018), and the CWD prion type in reindeer, even if displaying similarities with the North American CWD, are different from what has been reported in North America. Finland and Sweden have detected PrPSc in respectively one and 3 old moose, and the preliminary analyses revealed similarities with the CWD type of the Norwegian moose. Further research is ongoing to characterize the disease, especially the red deer, which shows some peculiar characteristics also distinct from reindeer and moose type. This suggests that multiple strains affect European cervids and that they are different from the ones described in North America.

Doctor **Elvan Boke** (Centre for Genomic Regulation (CRG)- Cell and development Biology- Leader of the Oocyte Biology and Cellular Dormancy group, Barcelona, Spain)

Doctor Elvan Boke graduated as valedictorian from Department of Molecular Biology and Genetics, Middle East Technical University, in Ankara, Turkey in 2008.

After pursuing a PhD in Cell Cycle in Cancer Research UK Manchester Institute (2008-2012), United Kingdom, she moved to Boston, United States of America, to perform her postdoctoral research at the department of Systems Biology in Harvard Medical School (2013-2016).

She is leading the Oocyte Biology and Cellular Dormancy group in Centre for Genomic Regulation (CRG) in Barcelona since 2017.

She is developing work to reveal the mechanisms dormant oocytes employ to remain viable; this research has implications both for the structure and function of vertebrate organelles and the regulation of physiological amyloid-like structures.

Prion and Prion-like diseases in humans

The Balbiani Body: A super-organelle held together by amyloid-like assembly

Most vertebrate oocytes contain a Balbiani body, a large, non-membrane-bound compartment packed with RNA, mitochondria, ER and Golgi. Little is known about this compartment, though it specifies germline identity in many non-mammalian vertebrates.
We showed that the Balbiani body is held together by a physiological amyloid network, which disperses upon maturation in Xenopus laevis (frog) oocytes. Velo1, a disordered protein with an N-terminal prion-like domain, is an abundant constituent of Xenopus Balbiani bodies. Disruption of the prion-like domain of Velo1, or substitution with a prion-like domain from an unrelated protein, interferes with its incorporation into Balbiani bodies in vivo. Recombinant Velo1 forms amyloid-like networks in vitro. Amyloid-like assemblies of Velo1 recruit both RNA and mitochondria in cell-free assays. We proposed that Xenopus Balbiani bodies form by amyloid-like assembly of Velo1 , accompanied by co-recruitment of mitochondria and RNA.
The link between the material state of the Balbiani body and its function remains unknown. I will present recent unpublished data from my group on the possible link between the physiological amyloid state of the Balbiani body and its function.
Prion-like domains are found in germ plasm organizing proteins in other species, suggesting that Balbiani body formation, and disassembly could be a conserved mechanism that helps oocytes function as long-lived germ cells.

Prion Structure and Biology

Oral Communications

Prion Structure and Biology

01

Unraveling Common Molecular Mechanisms of Prion Propagation

Giovanni Spagnolli[1], Luca Terruzzi[1], Alberto Boldrini[1], Jesús R. Requena[2], Pietro Faccioli[3,4], Emiliano Biasini[1]

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How does a prion propagate? Answering this question remains one of the most outstanding challenges in biology. Indeed, the high-resolution reconstruction of prion replication would illuminate the striking molecular mechanism underlying the phenomenon of protein-based inheritance. Unfortunately, this is not approachable experimentally because of the lack of atomistic, time-resolved biophysical techniques. Standard computational methods, like Molecular Dynamics (MD), would represent valid alternatives, but are currently limited by the enormous calculation load required to simulate such molecular paths. We recently addressed this problem by applying transition-sampling MD algorithms, techniques allowing to reduce the computational cost of simulating complex molecular events. We obtained the first atomistic reconstruction of the propagation of a 4-rung-β-solenoid (4RβS) mammalian prion[1]. While this work represents a pioneering attempt to study prion misfolding, it relied on three assumptions: (i) the initial contact point assignment between PrP^{C} and the templating surface of PrP^{Sc} (ii) the use of a computational model for the 4RβS architecture; (iii) the need for a statistical model to instruct the reaction progression. In this study, we overcame these limitations by applying an updated MD algorithm (called Self-Consistent Path Sampling) to simulate the propagation of the fungal prion Het-s, whose 2RβS architecture was solved by ssNMR[2]. We obtained an assumption-free atomistic reconstruction of Het-s replication, which showed remarkable similarities with the previously reported PrP^{Sc} misfolding mechanism. These results suggest that the propagation of prions generated by evolutionary distant proteins shares common molecular features that likely underlie their ability to propagate their conformation in a templated fashion.

References:
[1] Spagnolli G, et al. PLoS Pathog. 2019; doi: 10.1371/journal.ppat1007864
[2] Wasmer C, et al. Science. 2008; doi: 10.1126/science.1151839

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02

A Structural Insight of Infectious Recombinant PrP^{Sc} Using FTIR

Yaiza B. Codeseira[1], Phillip Pinder[2] , Sonia Veiga[1]; Hasier Eraña[3]; Joaquín Castilla[3] , Michael Beekes[2] , Jesús R. Requena[1]

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Understanding the PrP^{Sc} structure constitutes a key challenge in prion research. We have recently developed the first physically plausible model of PrP^{Sc}, featuring a four-rung β-solenoid, that agrees with all the experimental structural constraints available. However, another one has been proposed: the parallel in-register intermolecular sheet model (PIRIBS). In order to challenge such model we have compared two different PrP proteins using Fourier-transformation infrared spectroscopy: a recombinant, infectious *bona fide* PrP^{Sc}, and a non-infectious PrP PIRIBS amyloid. We prepared $recPrP^{Sc}$ using protein misfolding shaking amplification (PMSA) and designed an *in vivo* assay to prove that it was infectious material.
The second derivative FTIR spectrum of the non-infectious PrP amyloid showed characteristic absorption bands in the Amide I/II region, with a peak exhibiting a maximum at 1626 cm^{-1} and a shoulder at ~1630 cm^{-1}, typical of β strands, and a group of peaks with maxima at 1674 and 1663 cm^{-1}, ascribed to loops and turns. In turn, the spectrum of $recPrP^{Sc}$ exhibited a sharp peak with a maximum at 1632 cm^{-1}, that was slightly asymmetric, as if featuring an unresolved shoulder at a slightly lower wavenumber. Peaks with maxima at ~1674 and ~1663 cm^{-1} in the turn/coil region were also seen. The data clearly shows that infectious $recPrP^{Sc}$ and non-infectious recPrP amyloid exhibit distinctly different FTIR microspectroscopy spectra. This, in turn, suggests that these two conformers have structural differences. These results support the four-rung β-solenoid model.

A largely scalable new method to produce infectious recombinant prions

Hasier Eraña[1,2], Jorge M. Charco[1], Michele A. Di Bari[3], Carlos M. Díaz-Domínguez[1], Rafael López-Moreno[1], Enric Vidal[4], Ezequiel Gonz lez-Miranda[1], Miguel A. P rez-Castro[2], Sandra García-Martínez[1], Susana Bravo[5], Natalia Fern ndez-Borges[1], Mariví Geijo[6], Claudia D Agostino[3], Joseba Garrido[6], Jifeng Bian[7], Anna K nig[8,9], Boran Uluca-Yazgi[8,9], Raimon Sabate[10,11], Vadim Khaychuk[7], Ilaria Vanni[3], Glenn C. Telling[7], Henrike Heise[8,9], Romolo Nonno[3], Jesús R. Requena[12] and Joaquín Castilla[1,13]
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The resolution of the three-dimensional structure of infectious prions at the atomic level is pivotal to understand the pathobiology of Transmissible Spongiform Encephalopathies (TSE), but has been long hindered due to certain particularities of these proteinaceous pathogens. Difficulties related to their purification from brain homogenates of disease-affected animals were resolved almost a decade ago by the development of *in vitro* recombinant prion propagation systems giving rise to highly infectious recombinant prions. However, lack of knowledge about the molecular mechanisms of the misfolding event and the complexity of systems such as the Protein Misfolding Cyclic Amplification (PMCA), have limited generating the large amounts of homogeneous recombinant prion preparations required for high-resolution techniques such as solid state Nuclear Magnetic Resonance (ssNMR) imaging.

Herein, we present a novel recombinant prion propagation system based on PMCA that substitutes sonication with shaking thereby allowing the production of unprecedented amounts of multi-labeled, infectious recombinant prions. The use of specific cofactors, such as dextran sulfate, limit the structural heterogeneity of the *in vitro* propagated prions and makes possible, for the first time, the generation of infectious and likely homogeneous samples in sufficient quantities for studies with high-resolution structural techniques as demonstrated by the preliminary ssNMR spectrum presented here.

Overall, we consider that this new method named Protein Misfolding Shaking Amplification (PMSA), opens new avenues to finally elucidate the three-dimensional structure of infectious prions.

This study has been funded by MINECO research project references BFU2017-86692-P, BFU2013-48436-C2-1-P and RTI2018-098515-B-I00, and by RedPRION (Interreg POCTEFA EFA148/16).

A first bacterial extracellular and functional prion-like protein.

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Prions are proteins with the amazing capacity to switch between a soluble conformation and a self-perpetuating amyloid state. Initially associated with neurodegenerative diseases, it is now clear that prion-like mechanisms are exploited for functional purposes. Prion-like behaviour was thought to be restricted to eukaryotic organisms, but the discovery that *Clostridium* Rho terminator works as a prion broke this dogma. All prion-like proteins discovered so far are intracellular, mostly playing regulatory roles. Here, we describe the discovery and characterization of a first extracellular prion-like protein. This protein bears a characteristic disordered and low-complexity prion domain that drives the formation of amyloid fibrils and is indispensable for its function as a biofilm remodelling protein in *Staphylococcus aureus*. We show, for the first time, that a prion-like domain has evolved to play a role thought to be restricted to small globular domains: biofilm binding, a function that is directly connected with the ability of the bacteria to cause nocosomial infections.

Prion Diseases in Animals

Oral Communications

Prion Diseases in Animals

Broad study of the diversity of classical scrapie prions circulating in Europe by using just two rodent models

Alba Marín-Moreno[1], Patricia Aguilar-Calvo[1], Juan Carlos Espinosa[1], Lorenzo González[2], José Luis Pitarch[1], Diego Sola[3], Cristina Acín[3], Leonor Orge[4], Olivier Andreoletti[5], Romolo Nonno[6], Juan María Torres[1, *].

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5. UMR INRA ENVT 1225-IHAP, École Nationale Vétérinaire de Toulouse, Toulouse, France.
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Classical scrapie is often named as a uniform disease caused by a single prion strain. However, several scrapie strains producing different prion diseases phenotypes have been reported in the literature. A systematic analysis of the strains present in the small ruminant European herds has not been addressed.

Goat and sheep scrapie isolates deriving from several European countries were intracranially inoculated into transgenic mice overexpressing ovine and bovine PrP proteins (Ov-Tg501 and Bo-Tg110 respectively). Two iterative passages were performed. On the second passage, once overcome the transmission barrier, an accurate classification of the isolates was possible. In addition, the use of just two rodent models was enough to fully differentiate among the small ruminant TSEs diversity successfully identifying at least four different classical scrapie groups with different strain phenotypic features. In addition, mixtures of two strains were identified in a single isolate.

This work reinforces the idea that classical scrapie in sheep and goats is a prion disease caused by multiple different prion strains and not a single strain as is the case of epidemic Bovine Spongiform Encephalopathy (BSE).

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Mixtures of prion substrains in field cases of scrapie revealed by mice bioassay

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Phenotypic variability in prion diseases is associated to the existence of prion strains. The strain phenomenon can be accommodated within the protein-only hypothesis through the notion that different conformational variants of PrPSc encode distinct pathobiological properties. Within this framework, the conformational selection model proposes that a given PrP amino acid sequence allows a limited number of folding states, and that the degree of overlapping between PrPSc conformations in the inoculum and permitted conformations of host PrPC determines the strength of the transmission barrier. In the context of a heterotypic interaction, conformational selection may favor minor components from this “cloud” and thus modify the range of prion variants that propagate. Therefore, transgenic models expressing homologous PrPC are crucial to faithfully study the actual variety of prion strains. Ovinized mice show enhanced susceptibility to infection with scrapie prions and have been employed to characterize strains in natural sheep isolates.

In the present study, we used two ovine PrPC-expressing models to bioassay 20 sheep scrapie isolates from distinct outbreaks within the Spain-France-Andorra transboundary territory. Animals were intracerebrally inoculated and survival periods, lesion profiles, PrPSc distribution and banding patterns were studied.

Inocula showed a remarkable homogeneity on banding patterns, all of them but one showing 19-kDa PrPres. However, a number of isolates caused accumulation of 21-kDa PrPres in nervous tissue of TgShp XI mice while presenting survival periods and neuropathological features similar to the rest. A different subgroup of isolates caused very long survival periods with low attack rates and presence of 21-kDa PrPres in Tg338 mice. These animals also showed milder spongiosis and occasional presence of amyloid plaques.

These results suggest that some scrapie isolates contained mixtures of substrains that were resolved distinctly in each transgenic model. The major 19-kDa component and the two distinct 21-kDa components seemed to coexist in source inocula, as not all challenged mice developed the same phenotype. The reason why each transgenic model favors a specific component of the mixture is unknown, although PrPC expression level may play a role. Our results also indicate that coinfection of sheep with more than one scrapie strain is more frequent than infection with a single component.

07

Therapeutic assay with the non-toxic C-terminal fragment of tetanus toxin (TTC) in transgenic murine models of scrapie

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Tetanus neurotoxin is produced by Clostridium tetani, the causative agent of the tetanus. This neurotoxin enters neurons thanks to its non-toxic C-terminal fragment (TTC), which is the responsible for neuron binding, internalization and retrograde and transsynaptic transport. Therefore, TTC can be attached to therapeutic molecules and used for targeting them to the CNS. Moreover, TTC alone binds to Trk receptors and activates Trk-dependent signaling, mimicking the action of natural neurotrophic ligands in the activation of neuronal survival pathways and apoptosis inhibition.

We present here a pilot study to test the therapeutic potential of TTC to treat prion diseases. Two groups of Tg338 transgenic mice (overexpressing the VRQ variant of sheep PrP^{C}) were intracerebrally inoculated with scrapie isolates. Then, one of the groups (n = 5) was intramuscularly inoculated with TTC once a month, for six times, while the control group (n = 5) was intramuscularly inoculated with PBS following the same pattern. Mice were sacrificed after the onset of clinical signs. TTC-treated mice lived 16 days longer than control mice, this difference was statistically significant (Mantel Cox test, $p < 0.05$). Neuropathological changes were studied by hematoxylin-eosin staining and the distribution of PrP^{Sc} was evaluated by PET-blot. Moreover, immunohistochemical techniques were used for LC3β, p62, BAX, caspase-3 and NeuN detection in order to assess autophagic mechanisms, apoptosis and neuronal survival, respectively. Preliminary results show that TTC may have a neuroprotective effect in prion diseases affected mice, as it seems to increase neuronal survival and autophagic processes and decrease apoptosis in scrapie-infected mice.

08

Infectivity study: inoculation of tg340 mice with tissues from resistant goats inoculated with bovine and caprine bovine spongiform encephalopathy

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Transmissible spongiform encephalopathies affect both the human species and the animals, characterized by long incubation period and neurodegenerative lesions that lead to the death of the individual. The first cases of BSE in the United Kingdom and later in the rest of the EU, as well as the association of this disease with the vECJ has led to the adoption of numerous legislative measures aimed at eradicating BSE and preventing its transmission. In the case of scrapie, there is no evidence that it supposes a risk to human health. However, it has been shown that the goat species can also be affected by BSE in a natural way, which shows our main objective, the importance and the need for new studies to determine and evaluate the public health risk represented by goat products affected by BSE.

For this study, tg340 human transgenic mice, which express 4 times human PrP^{c} and are methionine for codon 129, have been used.

Samples of goat tissues infected with bovine BSE and goat BSE were used for the inoculums. In addition, each goat carried a genotype at codon 222: homozygous Glutamine / Glutamine (Q222Q), heterozygous Glutamine / Lysine (Q222K) and homozygous Lysine / Lysine (K222K).

Mice inoculated with goat brain around 750 days' post-inoculation presented clinical signs such as thinness, ataxia or lordosis.

By the immunohistochemical technique, the presence of PrP^{Sc} accumulations with different patterns was observed and by the Western Blot technique, glycosylation patterns compatible with BSE was observed.

09

Characterization of the first Portuguese cases of Atypical Scrapie in transgenic ovine ARQ-PrP mice

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Atypical Scrapie (ASc) is the dominant form of Transmissible Spongiform Encephalopathy (TSE) in small ruminants in Portugal. As a result of the EU active scrapie surveillance plan, ASc was diagnosed for the first time in 2003, making Portugal one of the first countries to ever report it. A total of 689 Portuguese AsC cases was confirmed until the end of 2018.

The first sheep isolates to be characterized included homozygous ARQ genotype and classical scrapie resistant homozygous ARR genotype. Those isolates- one of each genotype- were transmitted to TgshpXI mice expressing ovine PrP^{ARQ} (n =20) for atypical scrapie strain typing at Friedrich-Loeffler- Institute (German National Reference Laboratory for TSEs). The mean incubation periods were 414±58 days in mice inoculated with ARQ/ARQ genotype and 483±107 days in ARR/ARR genotype. Fixed, paraffin-embedded brains were sectioned at five reference coronal areas for histopathology and PrPsc immunohistochemistry (IHC). Lesional profiles were similar to French ASc Nor98 discordant cases, with vacuolation in regions G6, G8, W1 and W3 in both genotypes. IHC revealed aggregates and granular PrPsc deposition in the cerebellar cortex, hippocampus, cerebellar white matter and cerebral peduncles. Incubation periods, lesional profiles and PrPsc distribution were compatible with previously reported cases of ASc Nor98, transmitted to transgenic TgshpXI mice.

ASc has consistent pathology, PrPsc distribution, PrPsc electrophoretic profile in both naturally occurring cases and experimentally transmitted isolates. Considering the possibility of phenotype shift and the prevalence of this disease in Portugal, it is relevant to maintain these studies for evidence of any strain conversion.

10

Whole genome DNA methylation profiles in the central nervous system of sheep naturally infected with scrapie.

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Scrapie is a Transmissible Spongiform Encephalopaty (TSE) that affects sheep and goats and it is considered a good natural animal model to study prion diseases. Although changes in DNA-methylation occur in the Central Nervous System (CNS) in many neurodegenerative diseases, potential DNA-methylation alterations have not been investigated in any TSE models or naturally infected cases. We present here a whole genome sequencing analysis of bisulfite treated DNA (WGBS) obtained from thalamus of four naturally scrapie infected sheep and four controls. All animals were female, carried the ARQ/ARQ genotype for the PRNP allele and were sacrificed with similar age (4 to 6 years old). No differences in the genomic percentage of methylated cytosines (5mC) were observed between scrapie and control groups. Although genomes displayed similar average methylation levels, we identified 39 differentially methylated promoters (DMP) and a total of 8,907 differentially methylated regions (DMR). Gene Ontology enrichment revealed that hypomethylated DMRs were enriched in genes involved in transmembrane transport and cell adhesion whereas hypermethylated DMRs were related with intracellular signal transduction genes. The cellular prion protein (PrP^{C}) seems to act as an important regulator of cell adhesion and membrane barrier function. Therefore, the enrichment observed in these cellular processes when PrP^{C} has lost its function after the conversion to PrP^{Sc} could be indicative of an epigenetic regulation of these mechanisms. Moreover, a validation study using qPCR has shown differences in the expression of three genes (CD81, CABIN1 and SNCG) that match the methylation changes observed in the genomic study.

11

Susceptibility of human PrP Drosophila to cervid prions

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Chronic Wasting Disease (CWD) is an increasingly prevalent fatal transmissible prion disease that affects members of the cervidae family including deer, elk, moose and reindeer. The brain and peripheral tissues of CWD-affected cervids accumulate PrP^{Sc}, an abnormal conformer of the normal host protein PrP^{C}, which is considered to be the infectious prion agent. CWD has been detected in cervids in the USA and Europe, and poses significant challenges to human health because of its unknown zoonotic potential.

The molecular nature of the infectious prion agent remains undefined and the only reliable method to detect prion infectivity is by bioassay in a suitable experimental host. Attempts to address the zoonotic potential of CWD have involved inoculation of non-human primates, some of which have developed bona fide or atypical prion disease. These non-human primate CWD transmissions, some of which have taken up to10 years to perform, are incomplete as secondary transmissions are required. CWD prions have been inoculated into human PrP transgenic mice, which so far have shown resistance. Consequently, the zoonotic potential of CWD is unresolved and new experimental animal systems are required for this purpose.

To achieve this goal, we have generated human PrP Drosophila in order to model CWD permeability of the human species barrier. We have shown that human PrP Drosophila are susceptible to CWD isolates derived from the USA and Europe. Our data show that Drosophila can be used, in a rapid and efficient manner, to contribute to the understanding of the zoonotic potential of CWD.

12

Quantitating prion polymorphisms from heterozygous CWD-infected white-tailed deer.

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Chronic wasting disease (CWD) is the only prion disease naturally transmitted among farmed and free-ranging cervids. By 2019, CWD-infected cervids had been detected in 26 states, three Canadian provinces, South Korea, Norway, Finland, and Sweden. Cervid PrP^{C} has at least 20 polymorphic sites. Quantitating those polymorphisms in CWD PrP^{Sc} provides a measure of PrP^{Sc} formation efficiency. Even two signal sequence polymorphisms can be quantitated indirectly through PrP^{Sc}. Chymotrypsin, trypsin, or trypsin/chymotrypsin was used to digest cervid PrP, resulting in a set of 18 peptides spanning the 20 polymorphic sites. These peptides are suitable for a mass spectrometry-based multiple reaction monitoring (MRM) analysis. Seven of the 18 peptides do not contain polymorphisms, so they can be used as internal standards to quantitate the relative amounts of the other, polymorphism-containing, peptides. The calibration curves relating the area ratios of the MRM signals from polymorphism-containing peptides to the internal standard peptides were linear and had excellent correlation coefficients. Samples from heterozygous (G_{96}/S_{96} and Q_{95}/H_{95}) white-tailed deer orally dosed with homozygous (G_{96}/G_{96}) CWD were analyzed. The G_{96} polymorphism comprised 75 ± 5% of the total PrP^{Sc} from the G_{96}/S_{96} heterozygotes. The same polymorphism comprised only 25 ± 5% of the Q_{95}/H_{95} heterozygote's PrP^{Sc}. Heterozygous deer facilitate conversion of different PrP^{C} polymorphisms into PrP^{Sc}. This is significant for managing the spread of CWD. The relative amounts of the polymorphisms present in other heterozygous animal species and even humans can be quantitated using this approach.

13

Elk-PrP^C expression levels do not alter the Wisc-1 CWD strain properties and favors its selection from mixtures

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Chronic Wasting Disease (CWD) is an epidemic prion disease affecting cervid species from North America, South Korea, and Scandinavia. Host PrP^C sequence is one major factor of prion disease susceptibility. Elk and deer PrP^C differ at residue 226 (elk expressing E and deer expressing Q). This amino acid difference has been hypothesized to affect prion strain evolution, host strain selection and pathogenesis. Similarly, expression of the Q95H amino acid polymorphisms has been associated with emergence of the CWD strain H95$^+$ in white-tailed deer exposed to wt/wt (Q95G96) CWD prions (Wisc-1 strain).

Amino acid differences are, however, not the only drivers of prion strain selection. PrP^C gene dosage has also been implicated in the generation of strains. We hypothesized that elk PrP^C levels could result in modifications of the Wisc-1 CWD strain. We also tested the effects of PrP^C over-expression on strain selection from a previously characterized strain mixture (H95+ and Wisc-1).

To test this hypothesis, two mouse lines expressing different levels of elk PrP^C (tg-elk$^{+/+}$ and tg-elk$^{+/-}$ mice) were inoculated with the elk CWD2 strain, the white-tailed deer Wisc-1 strain and a mixture of H95+ and Wisc-1 strains. Transmission of Wisc-1 and its mixture with H95$^+$ produced similar vacuolation profiles and PrP-res glycotypes in both tg lines. Likewise, no differences were observed between first and second passages in tg-elk$^{+/+}$. PMCA analysis using S96 PrP^C as a substrate, which favors H95$^+$ strain selection, failed to detect the presence of this strain in exposed tg-elk mice. Our results show that the transmission of deer prions through hosts expressing E226-PrP^C did not alter the strain properties and indicates that Wisc-1 strain was preferentially selected in both tg lines.

14

Regional variation in the prion protein gene (PRNP) in wild European deer species

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Following the emergence of chronic wasting disease (CWD) in Norway there have been concerns that the disease may spread within Europe, as has been the case in North America. It is therefore important to understand the genetic diversity of European deer species, with reference to the prion protein gene (PRNP), which can be used to predict susceptibility to prion disease.

We have performed a comprehensive survey of the PRNP gene sequence of over 1000 British deer, including six of the most numerous free-ranging species present in the UK: red, roe, fallow, sika, muntjac and Chinese water deer. Samples were taken from existing DNA archives or from hunter-collected samples across the UK, the PRNP ORF amplified by PCR and sequenced via Sanger sequencing for analysis.

We have established the common PRNP sequence variants in British deer species, which based on comparison with PRNP sequence variants in North American cervids as well as experimental challenge data, leads us to conclude that the majority of British species would be susceptible to CWD. We identified non-synonymous polymorphisms in red deer at codons 98, 168, 226 and 247. We identified significant regional variation in genotype frequencies in red deer in seven different UK locations, and compared this with red deer from Norway and the Czech Republic. PRNP polymorphisms at P168S and I247L have only been identified in Scottish and Czech red deer respectively, and their effects on CWD susceptibility are unknown.

Chronic wasting disease risk assessment in Portugal - Genetic variability preliminary results and future perspectives.

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Chronic Wasting Disease (CWD) belongs to the family of Transmissible Spongiform Encephalopathies (TSEs), specific to cervids, and characterized by an infectious, misfolding of the prion protein (PrP^{C}) into a protease-resistant form (PrP^{Sc}). Originated and widespread in the North America, the presence of this prion disease is nowadays recognized in 25 states of the USA, Canada, South Korea and, in 2016, reached Europe through Norway. Red deer (Cervus elaphus), roe deer (Capreolus capreolus) and fallow deer (Dama dama) are three of the cervid species found in Portugal. Although there hasn't been reported any positive case of CWD in Portuguese populations, examples of TSEs outbreaks in bovine, sheep and goats together with of co-habitation areas between these species, suggest that caution should be taken once exposure and contact with prions can occur. The study of susceptibility/resistance of cervids to CWD is essential to define its risk of dissemination/development as well as its potential as prion reservoir. The estimation of genetic variability in the prion protein (prnp) gene is one of the methodologies used to predict that certain populations are less susceptible to infection than others. In this way a synergistic collaborative project (Project 029947IC&T 02/SAICT/2017-SAICT) was established between the University of Trás-os-Montes and Alto Douro (UTAD), the National Institute for Agricultural and Veterinary Research (INIAV) and the Polytechnic Institute of Castelo Branco (IPCB) with the aim of evaluating the risk of a potential occurrence of CWD in cervid Portuguese populations. Here we present for the first-time preliminary results about the genetic variability of CDS region prnp gene in Cervus elaphus and Dama dama individuals from Portugal.

Prion and Prion-like diseases in humans

Oral Communications

Prion and Prion-like diseases in humans

16

Three pathological consequences of TACE α-secretase deregulation in prion diseases

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Physiologically, the α-secretase TACE (ADAM17) plays a protective role at the neuronal cell surface by catalyzing the cleavage of (i) TNFα receptors (TNFRs), conferring normal sensitivity to TNFα, (ii) the cellular prion protein PrP^{C}, which limits its conversion into pathogenic prions PrP^{Sc}, and (iii) the amyloid precursor protein (APP), which precludes the production of neurotoxic Aβ peptides. We assessed whether deregulation of TACE would contribute to neurodegeneration in prion diseases. Combining *in vitro* and *in vivo* approaches, we show that prion infection causes a deficit of TACE activity at the surface of neurons. Mechanistically, PrP^{Sc} promotes the overactivation of the kinase PDK1, which triggers the internalization of TACE. This diverts TACE activity away from TNFRs, sensitizing neurons to TNFα-associated inflammation. The neutralization of TACE also disrupts PrP^{C} α-cleavage amplifying the production of PrP^{Sc}. Finally PDK1-mediated TACE internalization leads to the accumulation of Aβ40/42 peptides that are produced as monomers but also as trimers and tetramers. Those Aβ peptides deposit in the brain of prion-infected mice, only when seeds of Aβ trimers are co-transmitted with PrP^{Sc}. Prion-infected mice with PrP^{Sc} and Aβ deposits display reduced survival compared to mice with PrP^{Sc} deposits only, indicating that the onset of a mixed PrP^{Sc}/Aβ pathology accelerates death of prion-infected mice. Rescuing TACE protective cleavage activity towards TNFRs, PrP^{C} and APP upon PDK1 inhibition mitigates prion diseases by desensitizing prion-infected neurons from TNFα toxicity and reducing PrP^{Sc} and Aβ amyloid loads. Altogether, our data posit PDK1 as a therapeutic option to combat prion diseases.

17

Plasma total prion protein as a potential biomarker for neurodegenerative dementia: diagnostic accuracy in the spectrum of prion diseases

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In the search for blood-based biomarkers of neurodegenerative diseases, we characterized the concentration of total prion protein (t-PrP) in the plasma of neurodegenerative dementias. We aimed to assess its accuracy in this differential diagnostic context.

Plasma t-PrP was measured in 520 individuals including healthy controls (HC) and patients diagnosed with neurological disease control (ND), Alzheimer's disease (AD), sporadic Creutzfeldt-Jakob disease (sCJD), frontotemporal dementia (FTD), Lewy body dementia (LBD) and vascular dementia (VaD). Additionally, t-PrP was quantified in genetic prion diseases and iatrogenic CJD. The accuracy of t-PrP discriminating the diagnostic groups was evaluated and correlated with demographic, genetic and clinical data in prion diseases. Markers of blood-brain barrier impairment were investigated in sCJD brains.

Compared to HC and ND, elevated plasma t-PrP concentrations were detected in sCJD, followed by FTD, AD, VaD and LBD. In sCJD, t-PrP was associated neither with age nor sex, but with codon 129 PRNP genotype. Plasma tPrP concentrations correlated with cerebrospinal fluid (CSF) markers of neuro-axonal damage, but not with CSF t-PrP. In genetic prion diseases, plasma t-PrP was elevated in all type of mutations investigated. In sCJD brain tissue, extravasation of immunoglobulin G and the presence of swollen astrocytic end-feet around the vessels suggested leakage of blood-brain barrier as a potential source of increased plasma t-PrP.

Therefore, plasma t-PrP is elevated in prion diseases regardless of aetiology. This pilot study opens the possibility to consider plasma t-PrP as a promising blood-based biomarker in the diagnostic of prion disease.

18

Optimising RT-QuIC for the detection of prion seeding activity in BSE-infected ovine blood.

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Attempts to develop a diagnostic test for variant Creutzfeldt-Jakob disease (vCJD) have been hampered by the extremely low titres of disease-associated prion protein (PrP^{Sc}) found in readily accessible biological samples, such as blood. Recently, several platforms have been developed which may be sensitive enough to overcome this issue, including the *in vitro* amplification method, 'real-time quaking induced conversion' (RT-QuIC).

Here we describe a novel version of the RT-QuIC assay that uses a previously untested recombinant prion protein (recPrP) to detect prion seeding activity in microliter volumes of whole blood. Due to a paucity of suitable human blood samples, we have optimised our assay using blood samples from sheep that have been experimentally infected with BSE; a well-established large animal model of vCJD infection. [1]

To overcome the inhibitory effects of whole blood, our RT-QuIC assay utilises a 'pre-cleaning' step, whereby the PrP^{Sc} in each sample is captured on iron oxide beads. [2] Using this methodology, we can accurately detect seeding activity in 'exogenously' spiked blood with a high degree of analytical sensitivity (equivalent to a 10^{-6} dilution of BSE-infected brain homogenate). Our assay has been further optimised to accurately detect seeding activity in 'endogenously' infected blood, collected from BSE-infected sheep at the onset of clinical signs. If this version of the RT-QuIC reaction demonstrates similar efficacy when applied to blood samples from preclinically infected animals, it could then be adapted as an early diagnostic test for vCJD in humans, or as a means of testing donated blood for prion infection.

[1] McCutcheon, et al. PLoS ONE 2011; 6(8): e23169
[2] Denkers, et al. J Gen Virol 2016; 97:2023-2029

19

Amino acid residues in β2-α2 loop of human-PrP regulate prion strain susceptibility

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Previous data evidenced the key role of the b2-a2 loop of PrP in prion strain transmission barriers. Recent results from our group comparing the susceptibility to several prion strains in transgenic mice expressing either macaque or human-PrP^{C} support that two amino acid differences in the b2-a2 loop observed between macaque and human-PrP sequences (V166 and Q168 in macaque or M166 and E168 in human) are relevant for the susceptibility/resistance to prion strains such as classical-BSE. For these reasons, the susceptibility to a panel of different prion strains has been compared in VDQ-HuTg372 transgenic mice expressing human-PrP^{C} with M166 to V166 and E168 to Q168 amino acid changes ($_{166}$VDQ$_{168}$ human-PrP^{C}) and in MDE-Hu-Tg340 transgenic mice expressing wild type human-PrP^{C} ($_{166}$MDE$_{168}$ human-PrP^{C}). The results suggest that M166 and E168 amino acids residues in the human-PrP sequence are key elements limiting the propagation of most of the assayed prion strains either reducing the attack rates or shortening the survival time of the infected animals. It should be noted that human-PrP is the only one primate-PrP sequence harbouring $_{166}$M and $_{168}$E amino acid residues. All together, these results suggest that the selective pressure for changes in PrP amino acid sequence reducing the susceptibility to potentially circulating prions could promote the evolutive selection of the $_{166}$M and $_{168}$E amino acid residues in the human-PrP sequence.

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sCJD agents distribution in peripheral tissues

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Sporadic CJD is the most frequent form of Prion disease in human. Based on PrP^{Sc} detection results, sCJD prion distribution is considered to be mostly restricted to CNS. In contrast in vCJD, an acquired form of prion disease caused by the ingestion of BSE agent, the presence of abnormal PrP has been detected in numerous peripheral tissues in affected patients

In this study we estimated by bioassay the levels Prion infectivity in a large panel of peripheral tissues collected from six MM1 sCJD and 4 vCJD affected patients.

Unexpectedly we observed that in both sCJD and vCJD, infectivity can be found in numerous peripheral tissues in sCJD affected patients (like heart, kidney, lung, lymphoid organs or even salivary gland).

Although strong inter-individual variations exist between sCJD affected patients, the infectivity level observed in sCJD patients' peripheral tissues were not dissimilar from those observed in vCJD patients.

At this stage how early before clinical onset sCJD agent could accumulate in peripheral tissues remains unknown. However, our finding strongly supports the view that the iatrogenic transmission risk that is associated to peripheral tissues in sCJD should not be disregarded.

vCJD strain is consistent in individuals of two *PRNP* codon 129 genotypes

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The first clinical case of definite variant CJD in a *PRNP* heterozygous individual was reported in 2016 and raised concerns over whether 129MV individuals can transmit the vCJD infection and whether the 129MV genotype could give rise to a novel strain of vCJD. We have now undertaken the *in vivo* strain characterisation of both a clinical case of vCJD and that of an asymptomatic case of vCJD in 129MV individuals.

Primary passage of the clinical case of 129MV vCJD in wildtype mice gave rise to pathologically confirmed (TSE vacuolation and PrP deposition) clinical disease. Differences in transmission data were observed at the primary and first subpassage of the asymptomatic 129MV case compared to 129MM transmissions. These differences included low attack rates with no or little TSE vacuolation at primary passage and changes in incubation periods and rankings. These differences were resolved upon further passage giving rise to strain characteristics consistent with 129MM vCJD and BSE transmission data.

These studies comprise the first strain characterisation of vCJD in 129MV individuals. Upon primary passage of the clinical case of 129MV vCJD transmission characteristics resemble that of the129MM vCJD and BSE strain. While some differences were observed at primary and subpassage of the asymptomatic case, these resolved by the second subpassage and strain characteristics are totally consistent with 129MM vCJD. We demonstrate that vCJD strain properties are not affected by transmission through individuals with the 129MV genotype and thus no alteration in virulence should be associated with different host genotype.

Prion Structure and Biology

01

The cellular prion protein fine-tunes the expression of E-cadherin through TGFβ signaling

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Cellular prion protein (PrP^C) which is well-known for its implication in prion diseases, emerges as a key protagonist of stem cell homeostasis and differentiation. Depletion of PrP^C in zebrafish embryos provokes gastrulation arrest caused by down-regulation of E-cadherin and loss of tissue cohesion. The mechanisms by which PrP^C controls E-cadherin expression however remain enigmatic.

To address this issue, we exploit the murine 1C11 neuronal stem cell line that can convert into serotonergic or noradrenergic neurons. The 1C11 cell line endogenously expresses PrP^C at comparable levels whatever the differentiation state. Chronic silencing of PrP^C in 1C11 cells (PrP^{null}-1C11) was shown to impair neuronal differentiation. As for PrP-depleted zebrafish embryos, we show that PrP^{null}-1C11 cells display reduced level of E-cadherin at mRNA and protein levels. Decreased E-cadherin expression in the absence of PrP^C is associated with an increased expression of N-cadherin. We provide evidence that deregulation of Transforming Growth Factor-beta (TGFβ) signaling is a the root of E-cadherin/N-Cadherin variation in PrP^{null}-cells. We measure a rise in TGFβ level in the culture medium of PrP^{null}-1C11 cells vs 1C11 cells. Conversely, exposure of PrP^C-expressing 1C11 cells to exogenous TGFβ reduces the amount of E-cadherin in favor of N-cadherin, thus mimicking the PrP^{null} situation. Moreover, an increased amount of TGFβ receptor I (TGFβ-R1) and an excessive coupling of TGFβ-R1 to its downstream effector Smad3 are also recorded in PrP^{null}-1C11 cells. This study unveils that PrP^C balances the expression of E-cadherin and N-cadherin through TGFβ signaling, a PrP^C role possibly critical for neuronal differentiation and morphogenesis.

02

Spheroid cell culture as an innovative model for studies of prion infection and cellular prion protein function

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Prion infection is associated with a conformational transformation of the cellular prion protein to its abnormal, partially proteolytically resistant PrP^{TSE} variant. To investigate the physiological role as well as the pathogenesis of prions, exponentially growing cell cultures are widely used. However, their benefit is limited because of the absence of toxic effect of PrP^{TSE} accumulation and different physiology of cells grown in the 2D monolayer compared to tissues in the living organism. The aim of this study is to develop a new 3D model of differentiated cells that will better simulate common conditions in the nerve tissue.

For this purpose, a mouse neuronal CAD5 cell line was selected based on its ability to be infected with prions and also stimulated to differentiate in vitro by change of culture medium and serum withdrawal. Furthermore, the control CAD5KO cell line with a deleted gene encoding the cellular prion protein was created using CRISPR/Cas9 genome editing. Cultivation of CAD5 cell suspension under constant rocking resulted in formation of spherical multicellular aggregates, so-called spheroids. The elimination of serum from the cell culture medium allowed the long-term cultivation of spheroids for at least six weeks. Concurrent incubation of the cells with an infectious brain homogenate enabled subsequent detection of prion infection not only by Western blot, but also immunohistochemically on spheroid cross sections.

Our data indicate that CAD5-derived spheroids represent a valid model that could complement other tools generally used in the prion research field.

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Prion Diseases in Animals

Scientific Posters

Prion Diseases in Animals

Neurofilament in blood: a new promising biomarker of scrapie

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Introduction. The availability of a manageable, early, and trustworthy test in prion disorders is a crucial issue for public health reasons, but it is still missing. While cerebrospinal fluid (CSF) biomarkers at least for Creutzfeldt-Jakob disease (CJD) are officially recognized, no blood biomarkers are available. Neurofilament light chain (NfL) levels represent a surrogate biomarker of neuroaxonal degeneration. To date, CSF and/or blood NfL L in prion diseases have been investigated in a few studies, disclosing high NF-L levels in CJD and other neurodegenerative disease patients. Interestingly, a successful use of the SIMOA ultra-sensitive single molecule array technology to test for NF-L concentrations in blood plasma or serum in human preclinical stages of CJD was reported. No studies are available regarding NfL concentrations in healthy or neurological diseased animals. Here, we highlight a promising utility of blood serum NF-L as biomarker of scrapie in sheep.

Materials and Methods. A set of 20 sheep blood serum blinded samples have been tested by the SIMOA technology. Nine samples originated from scrapie affected sheep (2 symptomatic animals) and 11 from healthy animals. The gold standard was assessed by testing the *medulla oblongata* of sheep both by an EU approved Elisa rapid test and by a Western blot assay.

Results. Results revealed a clear capacity of Simoa NfL test to classify between the two groups of samples according to the presence or absence of scrapie. The median serum NfL concentration in scrapie animals was more than 15 times higher than that found in control samples. However, the serum NfL concentration in scrapie sheep with clinical signs (n=2; 75.3, 15.7 pg/ml) did not significantly (p=0.541; t-test) differ from scrapie animals without clinical signs (n=7; 61.0, 10.7 pg/ml). It is of note that all control animals but one had serum NfL concentration well below the lowest level of scrapie sheep.

Conclusion.

Our data show that scrapie-affected sheep have significantly higher levels of serum NfL than control sheep, similarly to what has been reported for human TSEs and other patients affected by degenerative or inflammatory diseases of the nervous system. Thus, the use of the Simoa test for measuring serum NfL makes progress in the diagnosis of scrapie in living sheep. Moreover, the finding that blood samples of asymptomatic scrapie-affected sheep had serum NfL levels similar to those observed in clinically affected sheep suggests that serum NfL increases very early in the disease process and that the Simoa test is a quick and efficient tool for screening the entire sheep population in flocks at risk for scrapie with considerable economic benefits.

Underestimation of the scrapie prevalence in a goat herd by using a rapid diagnostic test

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Current European scrapie regulations require the testing of a proportion of dead animals, and outline measures in the case of disease confirmation. An outbreak of classical scrapie in a goat herd led to the culling of the whole herd, providing the opportunity to examine a subset of 151 goats, take samples and examine them for the presence of disease-associated prion protein (PrP^{Sc}) to inform on scrapie test sensitivity, pathology and association with prion protein genotype.

Goats were examined clinically prior to culling, and the brains examined by BioRad ELISA and two confirmatory tests, Western blot and immunohistochemistry. Furthermore, up to ten lymphoid tissues were examined by immunohistochemistry. Three goats (2.0%) tested positive for scrapie by ELISA and confirmatory tests, and a further five (3.3%) were negative by ELISA but positive by at least one of the confirmatory tests. Only two of these, both positive by ELISA, displayed evident clinical signs of scrapie. In addition, ten (6.6%) goats, including two clinical suspects, were negative on brain examination but had PrP^{Sc} in lymphoid tissue, most frequently in the medial retropharyngeal lymph node (94.4% of all 18 cases) and palatine tonsil (88.9%). Significantly more scrapie-positive goats were isoleucine homozygous at codon 142 (II_{142}) compared to methionine carriers (IM or MM_{142}).

The results indicate that, in goats, the sensitivity of the screening test ELISA is poor compared to the gold standard confirmatory tests, particularly for subclinical animals. Surveillance sensitivity could be improved by testing retropharyngeal lymph node or palatine tonsil in addition to brain.

05

Dogs are resistant to prion infection. Amino acid in position 163 key of such extreme resistance

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Unlike other species, such as cattle, cats or humans, prion disease has never been described in dogs, which prompted a thorough analysis of the dog *PRNP* gene. The presence of a negatively charged amino acid in position 163 was readily identified as a potential key to this resistance since it was the one differing form all other susceptible species. In fact, recent results from our group demonstrate that introducing this canid substitution in the PrP of mouse and bank vole causes a dramatic reduction of their susceptibility to prion infection.

In the present study a transgenic mouse model was generated expressing dog prion protein (with glutamic acid at position 163) and was challenged intracerebrally with a panel of prion isolates (including cattle BSE, sheep scrapie, atypical sheep scrapie and CWD among others) none of which could infect them. The brains of these mice were further subjected to *in vitro* prion propagation by PMCA ruling out the presence of undetected minimal amounts of prions and providing hard experimental evidence of dogs' suspected resistance to prions. Subsequently, a second transgenic model was generated in which asparagine (the most common amino acid in prion susceptible species) was introduced in position 163, which yielded this model susceptible to BSE-derived isolates.

This findings strongly support the hypothesis that glutamic acid in position 163 of the PrP^{C} is a major determinant of the extreme resistance of the canidae family to prion infection and establish this amino acid position as a promising therapeutic target for prion diseases.

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06

The impossibility to induce misfolding of chicken PrP suggests that prion diseases may be restricted to mammals

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Prion diseases have been reported in numerous species since their initial description in small ruminants. However, all the species confirmed to be susceptible to transmissible spongiform encephalopathies belong to the class Mammalia, with highly conserved PrP sequences. Nonetheless, genes equivalent to the mammalian *PRNP* have been found in the closest classes, birds and reptiles, with around 30 % sequence identity. Although the sequence identity is low when compared to the mammalian PrP, the molecular architecture of these proteins is highly similar and conserves almost all secondary structures, with differences mainly in α-helix 1 and the loops connecting them. Since prions are encoded in the tertiary structure of the misfolded PrP, assessing whether avian PrP could be misfolded into an infectious counterpart is of high interest due to relevance of poultry farming for human consumption.

We choose chicken PrP as a model of avian prion protein and unprecedented efforts were done to achieve chicken PrP misfolding and prion infection. Serial PMCA were performed using chicken brain homogenates seeded with a panel of prion isolates and using recombinant chicken PrP. Transgenic mouse lines were also generated overexpressing chicken PrP (TgChk) and used for PMCA and also challenged *in vivo* up to three passages. Actual chicken were also inoculated with BSE isolate performing a second passage in TgChk. However, in spite of the titanic efforts done, no signs of prion disease or PrP^{res} were detected in any case, proving to the extent possible that prion diseases could be restricted to mammals.

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07

Analysis of Toll-like receptors in ileum of orally inoculated lambs with classical scrapie

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Common pathological phenotypes found in prion diseases and other neurodegenerative disorders could be explained by uncontrolled chronic immune and inflammatory responses. Most cell types in central nervous system express Toll-like Receptors (TLRs), which are able to recognize damage-associated molecular patterns (DAMPs) of endogenous origin and trigger an innate immune response. TLRs have been found up-regulated in other neurodegenerative diseases and specifically recPrP has shown to induce certain TLR response. In animal transmissible spongiform encephalopathies, the oral route is assumed to be the natural prion entry. The mechanisms by which infectious prions cross the gut remain elusive but TLRs expressed by enterocytes and gut associated lymphoid cells might be involved in the prion uptake.

We analyzed mRNA expression of the TLRs and 3 cytokines (TNFα, TGFβ and IL-10) in ileum of lambs orally inoculated with ovine scrapie. Inoculations were performed 2 days after birth with 1 g of scrapie-infected (infected group, n=14) or non-infected (control group n=8) sheep brain and the animals were euthanized 48 hours post inoculation. TLR7 and TNFα were significantly increased in lambs of the scrapie-infected group. Previous studies have establishedthatTLR7 activation induce the release of TNFα by dendritic cells (DCs), which are decisive for the prion transport from gut epithelium to lymphoid associated tissues in scrapie pathogenesis. Moreover, TNFα is one of the central cytokines in DCs migration and activation. Further studies are necessary to determine the cell types within the ileum that show increased TLR7 and TNFα protein expresión.

08

TgShM112I mouse, a unique model for a spontaneous, transmissible and atypical ovine prion disease

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Modelling atypical prion diseases, which arise spontaneously in animals, is challenging and is usually done through inoculation of natural isolates. But models of spontaneous atypical prion disease in animals were not available until now.

Transgenic mice overexpressing the M112I polymorphism of the gene encoding for the sheep cellular prion protein (1.5x compared to sheep brain levels) manifest clinical signs of a spontaneous spongiform encephalopathy at as early as 400 days of age. The brains of these animals show the pathological hallmarks of prion disease: spongiform change, astro and microgliosis and PrP^{res} deposits. On Western blotting an atypical 5 band PrP^{res} pattern resembling Nor98 atypical scrapie cases is observed.

Brain homogenates from spontaneous prion affected sick TgShM12I mice were inoculated in several models to assess its infectivity and characterize the prion strain generated: TgShM112I (ovine M112I ARQ PrP^{C}), Tg338 (ovine wt VRQ PrP^{C}), Tg501 (ovine wt ARQ PrP^{C}), TgBo110 (bovine PrP^{C}), TgBoM120I (bovine M120I PrP^{C}), Tga20 (murine wt PrP^{C}), TgVole (bank vole M109I PrP^{C}), bank vole (M109I PrP^{C}) and sheep (ARQ PrP^{C}). Blood (buffy coat), skeletal muscle and splenic tissue were also inoculated to assess the presence of peripheral prion infectivity in this model.

In this poster the preliminary results of the aforementioned bioassays are discussed leading to the initial conclusion that the strain spontaneously generated in this model is analogous to the one causing small ruminant atypical scrapie (Nor98). We thus present the transgenic mouse line TgShM12I line as a unique model for a *bona fide*, spontaneous, transmissible, atypical (Nor98-like) prion disease.

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09

Assessment of host immune response in natural scrapie after chronic dexamethasone treatment

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Neuroinflammation has been correlated with the progress of neurodegeneration in many neuropathologies. Although glial cells have been traditionally considered to be protective, the concept as neurotoxic cells has recently emerged. Thus, a major unsolved question now is the exact role of astroglia and /or microglia playing in neurodegenerative disorders. On the other hand, it is well known that glucocorticoids are the first choice to regulate inflammation and consequently, neuroglial inflammatory activity.

The objective of this study was to determine how chronic dexamethasone (DEX) treatment influences the host immune response in order to finally contribute to characterization of beneficial or detrimental role of glial cells at clinical stage of natural ovine Scrapie. With this aim, immunohistochemical expression of glial markers further PrP^{sc} accumulation, histopathological lesions and clinical evolution were comparetively assessed to control group.

Results provided here emphasize the complex interaction between glial populations, probably by means of cytokines released by both of them, specially dealing with a natural model. In addition, it intends to show whether modulation of neuroinflammation by antiinflammatory drugs may be converted into a research focus as a potential therapeutic target for prion diseases, in the similar manner that it is considered by some authors for prion-*like* diseases.

10

Genetic approach to the sequence of the *prnp* gene in Portuguese cervids species

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Chronic Wasting Disease (CWD) is a neurodegenerative pathology specific to *Cervidae* family, belonging to Transmissible Spongiform Encephalopathies (TSEs) and characterized by the deposition of prions – proteins with an aberrant conformation of a glycoprotein of the cells surface (PrPC).

A collaborative project (Project 029947IC&T 02/SAICT/2017-SAICT) was established with the purpose of assessing the risk of a potential CWD occurrence in Portugal, determining the *prnp* gene variability in the cervids population and comparing with the data from other countries. In order to characterize a partial sequence of the *prnp* gene in the Portuguese cervid population, new putative primers were designed to obtain a larger amplicon containing the internal region of intron 2 and the coding sequence (CDS) region, based on an alignment between several cervids. Until now, 50 samples from Portuguese red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) were used to perform the DNA extraction, followed by PCR amplification and Sanger sequencing, showing in first instance four polymorphic sites in the red deer (according to GenBank accession: MK103027.1).

This study will contribute to the increase of *prnp* gene knowledge and ultimately it will help in defining the risk associated with CWD in Portugal and Europe.

11

Genetic variability of the *PRNP* gene and resistance to *Scrapie* in Churra do Campo sheep breed.

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Classical *Scrapie* in sheep shows a pattern of genetic resistance related to polymorphisms in the prion protein gene (*PRNP*), leading to different amino acids (AA) codification at codons 136 (Alanine A /Valine V), 154 (Arginine Q/ Histidine H) and 171 (Glutamine Q/R/H). Resultant haplotypes/alleles are classified according to codded AA, and genotypes can be categorized in five degrees of resistance, from 1, more resistant (ARR/ARR), to 5 more susceptible (VRQ/VRQ). At EZN Molecular Genetics Laboratory (INIAV), *PRNP* is genotyped since 2003, using the SNaPshot ThermoFisher Scientific technique. This work aimed to identify the variability of *PRNP* locus at Churra do Campo Portuguese sheep breed. It was a collaboration between INIAV and the breed Herd Book, supported by the PDR2020 program and ALTBiotech$^{RepGen.}$ Project. This autochthonous breed underwent a recovery program since 2007 and currently has 597 females and 36 males, distributed by 6 breeders, being a genetic resource with great need of protection. A total of 530 samples of 7 farms were analyzed from year 2009 to 2019. Four alleles were found, being ARR, the predominant (0.548), followed by the ancestral ARQ (0.434). The most susceptible allele VRQ exhibits low frequency (0.011) and ARH only residual (0.007). Among the seven genotypes found, ARR/ARQ was predominant, (0.51), followed by the most resistant ARR/ARR (0.29) and ARQ/ARQ (0.17). These findings indicate that only 29% of the population studied can be included in the highest degree of resistance to classical scrapie, while the remaining has only an intermediate to low degree of resistance.

Key words: Scrapie, sheep

12

Role of ovine Ala136 and Arg154 polymorphisms in the conversion of PrP^C into PrP^{res}

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Scrapie Susceptibility in sheep is defined by amino acid polymorphisms in the ovine prion protein gene *Prnp*, particularly at positions 136, 154 and 171.

Most frequent genotypes in wild type animals, are those carrying both, Ala136 or Val136 polymorphisms, although Thr136 have been also found, meanwhile, at residue 154 Arg, His or Leu are present.

Although highest susceptibility is linked to $V_{136}R_{154}Q_{171}$/ $V_{136}R_{154}Q_{171}$ genotype, sheep carrying $A_{136}R_{154}Q_{171}$, $A_{136}H_{154}Q_{171}$ alleles are also susceptible to Scrapie to varying degrees. While Arg171 renders sheep resistant to Scrapie and BSE, pointing out that this residue is of paramount importance in defining the degree of susceptibility to disease, no resistance can be associated to Ala136 and Arg154 polymorphisms, suggesting a modulatory role of these residues in the resistance phenomenon to the disease.

Protein Misfolding Cyclic Amplification (PMCA) technique mimics the prion replication process but with accelerated kinetics. The recent development of the recombinant-PMCA (rec-PMCA) allowed us to change the source of PrP^C from brain tissue to recombinant protein, becoming an easier and cheaper way to study the role of polymorphisms affecting the Scrapie susceptibility.

The main goal of this study is to assess the role of the ovine PrP amino acid located at position 136 and 154 in the PrP^C into PrP^{res} in vitro conversion. Serial rec-PMCA experiments were designed to check the effect of the charge, size and/or hydrophobicity of distinct residues in these polymorphic residues as well as the thermal stability for residue 136 and 154 mutants.

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Prion and Prion-like diseases in humans

Cellular prion protein transcriptional regulation by tau in Alzheimer's disease

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Cellular prion protein (PrP^C) is the main responsible for prionopathies when it becomes the abnormally processed form (PrP^{SC}) and acquires self-aggregation, spreading and infective properties [1]. In the other hand, physiological PrP^C has protective functions against oxidative stress and excitotoxicity in neurons [2]. At initial stages of Alzheimer's disease (AD) an increase on PrP^C occurs [3], coinciding with ROS generation and accumulation of misfolded proteins; tau and β-amyloid [4]. It has been reported that ROS activates PrP^C transcription [5, 6]. However, the factors that upregulate PrP^C in AD are unknown.

For this reason, we studied the human PrP^C gene (PRNP) promoter under three hallmarks of AD in order to reveal the molecular mechanism involved in PrP^C overexpression. We used β-amyloid, tau protein, and ROS treatments to examine their specific roles in PRNP transcription regulation. In addition, we analyzed different transcription factors and signaling pathways involved in PRNP transcription control.

Our results showed PRNP transcriptional activation under tau treatment, independently of β-amyloid and ROS. The overexpression or uptake of non-fibrillar variants of tau protein enhanced PRNP activity. Finally, we determined AP-1 to be a transcription factor mediating the effects of tau.

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PrPsc-induced PDK1 overactivation promotes production of seedable amyloid-beta peptides in prion diseases.

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The occurrence of Alzheimer-like pathology in the brain of some CJD or GSS patients as well as individuals with iatrogenic CJD suggest potential interrelationship between prion pathogenesis and production and/or deposition of Amyloid-beta (Aß) peptides. Exploiting prion-infected neuronal cells and mice, we show that PrP^{sc}-induced overactivation of the PDK1 kinase and downregulation of TACE a-secretase activity shift the amyloid precursor protein APP towards its ß-processing and Aß40/42 overproduction. We found that Aß40/42 accumulates mainly as monomers but also as trimers and tetramers. To address the role exerted by the overproduced Aß on prion pathogenesis, we built an Aß-free prion infected cell system (referred to as PrP^{sc}-APP^{null} cells). The absence of Aß does not alter prion replication in PrP^{sc}-APP^{null} cells and cell-based PrP^{sc} inocula free or not of Aß display similar prion infectivities when injected to C57Bl/6J mice. We further show that PrP^{sc} is sufficient to promote a rise of Aß monomers and to generate Aß multimers in prion-infected mice whatever the presence of Aß in the PrP^{sc}-inocula. With the help of APP23 transgenic mice we provide evidence that PrP^{sc}-induced Aß display seedable properties as they can deposit in the mouse brain only when seeds of Aß trimers are co-transmitted with PrP^{sc}. Importantly, we show that brain Aß deposition accelerates death of prion-infected mice. Our data indicate that PrP^{sc}, through deregulation of the PDK1/TACE/APP pathway, provokes the accumulation of seedable Aß, a prerequisite for Aß deposition induced by an exogenous Aß seed and the onset of an Alzheimer-like pathology within a prion infectious context.

15

Removal of PrP^C-attached glycans favors the propagation and transmission of human strains with atypical characteristics

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Transmissibility is a requisite for a misfolded protein to be considered a prion. However, prion strains with atypical features, such as those responsible for GSS or VPSPr, have been historically endorsed with limited capacity of transmitting to other hosts. Interspecies transmission of prions is fundamentally modulated by the genotypic resemblance between PRNP sequences of donor and host. However, other factors are known to play a key role as well, including post-translational glycosylation of PrP^C. This work explores the relationship between PrP^C glycosylation and atypical features by studying the effect of glycan removal on the propagation and transmission of non-classical strains. Several human prion isolates were subjected to PMCA on a substrate prepared from brains of TgNN6h mice, which express human PrP^C with point mutations blocking glycosylation sites. Both direct and PMCA-adapted prions were later inoculated in TgNN6h and Tg340 mice, and neuropathological hallmarks were analyzed. Non-glycosylated human PrP^C was able to sustain in vitro propagation of different atypical human prions. The expression of this unglycosylated PrP^C in mice rendered them susceptible to experimental infection with either direct or in vitro-adapted atypical prions, including the poorly transmissible GSS A117V strain. Neuropathological features were preserved, indicating that bona fide replication of strain properties was achieved. In contrast, the GSS 6OPRI isolate could be propagated in both unglycosylated and normally glycosylated PrP^C-expressing models, which manifested distinct strain features, indicating a possible mixture of classical and atypical components. Our results suggest that the abolishment of PrP^C glycosylation facilitates the selective propagation of atypical strains, with maintenance of their specific pathobiological properties.

16

Viability of continuous intracerebral infusion of a tetrapyrrole in mice a possible therapy for prion disease

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Iron 5,10,15,20-Tetrakis(1-methyl-4-pyridinio)porphyrin tetra (p-toluenesulfonate) (FeTMPyP) is a cationic tetrapyrrole that behaves like a chemical chaperone, binding native PrP^C, stabilizing its conformation and preventing its conversion into PrP^{Sc}. This compound inhibits the propagation of prions -independently of the strain- in vitro and in cellula; nevertheless, since it cannot cross the blood-brain-barrier, its use as a possible anti-prion treatment has been dismissed.

Although a direct intracerebral infusion of a compound could be seen as an invasive route of administration, if we consider the fatal prognosis of human prion diseases and the inefficiency of the current treatments, this measure could be considered as an alternative and the risk would be justified.

We have assessed the viability and the tolerability of a daily continuous intracerebral administration of 16 nmoles of FeTMPyP for 45 days, using Alzet® osmotic minipumps implanted in the lateral ventricle in mice; considering a total distribution throughout the brain and a clearance in the cerebrospinal fluid, we estimate a constant concentration of ~4μM in the brain. This concentration which has shown a potent anti-prion activity in cell culture, has been well tolerated by all the mice. Additional studies with an even higher dose (36 nmol/day, which should result in a FeTMPyP brain concentration of ~9μM) are ongoing, also with good tolerability at the time of submission of this abstract.

This study demonstrates the viability of treating mice with a relevant therapeutic dose of FeTMPyP by intracerebral infusion, and sets the foundations for efficacy studies using murine models of prion disease.

Author index

Oral Communications

Prion Structure Biology

01 Giovanni Spagnolli, Luca Terruzzi, Alberto Boldrini, Jesús R. Requena, Pietro Faccioli, Emiliano Biasini.

02 Yaiza B. Codeseira, Phillip Pinder, Sonia Veiga, Hasier Eraña, Joaquín Castilla , Michael Beekes and Jesús R. Requena.

03 Hasier Eraña, Jorge M. Charco, Michele A. Di Bari, Carlos M. Díaz-Domínguez, Rafael López-Moreno, Enric Vidal, Ezequiel González-Miranda, Miguel A. Pérez-Castro, Sandra García-Martínez, Susana Bravo, Natalia Fernández-Borges, Mariví Geijo, Claudia D'Agostino, Joseba Garrido, Jifeng Bian, Anna König, Boran Uluca-Yazgi, Raimon Sabate, Vadim Khaychuk, Ilaria Vanni, Glenn C. Telling, Henrike Heise, Romolo Nonno, Jesús R. Requena and Joaquín Castilla.

04 Susanna Navarro, Jaione Valle, Manuel Baño, Iñigo Lasa and Salvador Ventura.

Prion Diseases Animals

05 Alba Marín-Moreno, Patricia Aguilar-Calvo, Juan Carlos Espinosa, Lorenzo González, José Luis Pitarch, Diego Sola, Cristina Acín, Leonor Orge, Olivier Andreoletti, Romolo Nonno and Juan María Torres.

06 Tomás Barrio, Hicham Filali, Alicia Otero, Jessica Sheleby-Elías, Belén Marín, Enric Vidal, Juan María Torres, Martin Groschup, Olivier Andréoletti, Juan José Badiola and Rosa Bolea.

07 Marina Betancor, Óscar López-Pérez, Laura Moreno, Alicia Otero, Tomás Barrio, Juan José Badiola, Rosario Osta, Rosa Bolea and Inmaculada Martín-Burriel.

08 Diego Sola, Helen Caroline Raksa, José Luis Pitarch, Jan Langeveld, Alex Bossers, Belén Marín, Francis Barillet, Frédéric Bouvier, Eva Monleón, Rosa Bolea, Olivier Andreoletti, Juan José Badiola and Cristina Acín.

09 Mafalda Casanova, Carla Machado, Paula Tavares, João Silva, Christine Fast, Anne Buschmann, Martin Groschup and Leonor Orge.

10 Hernaiz , A., Sentre , S., Filali , H., López-Pérez, O., Sanz, A ., Zaragoza , P., Badiola , J.J., Bolea , R., Toivonen , J.M. and Martín-Burriel , I.

11 Raymond Bujdoso

12 Christopher J. Silva, Melissa L. Erickson-Beltran, Camilo Duque Velásquez, Judd M. Aiken, and Debbie McKenzie.

13 Alicia Otero, Camilo Duque Velásquez, Chiye Kim, Jeffrey Narayan, Judd Aiken and Debbie McKenzie.

14 Amy L. Robinson, Helen Williamson, Mariella E. Güere, Helene Tharaldsen, Karis Baker, Stephanie L. Smith, Sílvia Pérez-Espona, Jarmila Krojerová-Prokešová, Josephine M. Pemberton, Wilfred Goldmann and Fiona Houston.

15 Jorge C. Pereira, Nuno Gonçalves-Anjo, Estela Bastos, Sara Rocha, Luís Figueira, Ana C. Matos, Carla Machado, João Silva, Paula Mendonça, Paulo Carvalho, Paula Tavares, Carla Lima, Anabela Alves, Alexandra Esteves, Maria L. Pinto, Isabel Pires, Adelina Gama, Roberto Sargo, Filipe Silva, Fernanda Seixas, Madalena Vieira-Pinto, Leonor Orge and Maria A. Pires.

Prion and Prion-like diseases in humans

16 Juliette Ezpeleta, Vincent Baudouin, Zaira Arellano-Anaya, Mathéa Pietri, Anne Baudry, Stéphane Haik, Yannick Bailly, Jean-Michel Peyrin, Odile Kellermann, Jean-Marie Launay, Benoit Schneider.

17 Franc Llorens, Anna Villar-Piqué, Matthias Schmitz, Daniela Diaz-Lucena, Marie Wohlhage, Peter Hermann, Isabel Schmidt, Markus Glatzel, Jean-Jaques Hauw, Beata Sikorska, Pawel P. Liberski, Joaquim Riggert, Isidro Ferrer and Inga Zerr.

18 Charlotte M. Thomas, Sandra McCutcheon, Richard A. Blanco, Boon Chin Tan and Fiona Houston.

19 Juan Carlos Espinosa, Alba Marín-Moreno, Patricia Aguilar-Calvo, Patricia Lorenzo, Ana Villa, Irene Prieto and Juan María Torres.

20 A.Huor, JY.Douet, H.Cassard, S.Lugan, N.Aron, M.Arnold, J.Ironside and O.Andreoletti.

21 Abigail B. Diack, Aileen Boyle, Chris Plinston, Diane Ritchie, Emma Hunt, Matthew Bishop, Robert G. Will and Jean C. Manson.

Scientific Posters

Prion Structure Biology

01 Marjorie Alvarez Mejia , Zaira E. Arellano-Anaya, Anne Baudry, Hector Ardila-Osorio, Edward Malaga-Trillo and Benoit Schneider.

02 Zdenka Backovska Hanusova, Zuzana Fremuntova, Tibor Mosko, Radoslav Matej and Karel Holada.

Prion Diseases Animals

03 H. Zetterberg, E. Bozzetta, C. Casalone, C. Corona, D. Loprevite, MG Perrotta, MC Cavarretta K. Blennow, M. Pocchiari and D. Meloni.

04 Timm Konold, John Spiropoulos, Jemma Thorne, Laura Phelan, Louise Fothergill, Brenda Rajanayagam, Tobias Floyd, Beatriz Vidana, Judith Charnley and Marion Simmons.

05 Hasier Eraña, Enric Vidal, Natalia Fernández-Borges, Beatriz Parra, Belén Pintado, Manuel Sánchez-Martín, Jorge M. Charco, Montserrat Ordoñez, Miguel A. Pérez-Castro, Martí Pumarola, Candace K. Mathiason, Tomás Mayoral and Joaquín Castilla.

06 Hasier Eraña, Natalia Fernández-Borges, Alicia Otero, Cassard Hervé, Carlos Hedman, Ezequiel González-Miranda, Manuel Sánchez-Martín, Carlos M Díaz-Domínguez, Enric Vidal, Tomás Barrio, Beatriz Parra, Belén Marín, Tomás Mayoral, Juan J Badiola, Rosa Bolea, Olivier Andreoletti and Joaquín Castilla.

07 Mirta García, Beatriz Serrano,Rosa Bolea, Eva Monleón, Juan José Badiola and Ma Carmen Garza.

08 Enric Vidal, Manuel Sánchez-Martín, Sierra Espinar, Hasier Eraña, Jorge M Charco, Miguel A Pérez-Castro, Rafael López-Moreno, Montse Ordóñez, Claudia D'Agostino, Michele A. Di Bari, Juan M Torres, Vincent Béringue, Rosa Bolea, Juan J. Badiola, Jésus R. Requena, Olivier Andreoletti, Romolo Nonno, Martí Pumarola and Joaquín Castilla.

09 I.M. Guijarro, M. Garcés, B. Marín, A. Otero, T. Barrio, J.J. Badiola and M. Monzón.

10 Nuno Gonçalves-Anjo, Estela Bastos, Sara Rocha, Jorge C. Pereira, Luís Figueira, Ana C. Matos, Carla Machado, João Silva, Paula Mendonça, Paulo Carvalho, Paula Tavares, Carla Lima, Anabela Alves, Alexandra Esteves, Maria L. Pinto, Isabel Pires, Adelina Gama, Roberto Sargo, Filipe Silva, Fernanda Seixas, Madalena Vieira-Pinto, Leonor Orge and Maria A. Pires.

11 Maria Fátima Santos-Silva, Alexandra S., Conceição O. Sousa, Carlos R. Andrade, Inês Carolino and Nuno Carolino.

12 Beatriz Parra, María J. Cano, Natalia Fernández-Borges, Enrique Anadón and Tomás Mayoral.

Prion and Prion-like diseases in humans

13 Laia Lidón, Cristina Vergara, Isidre Ferrer, Félix Hernández, Jesús Ávila, José Antonio del Río and Rosalina Gavín.

14 Zaira E. Arellano-Anaya, Juliette Ezpeleta, Vincent Baudouin, Mathéa Pietri, Anne Baudry, Anne-Marie Haeberlé, Yannick Bailly, Odile Kellermann, Jean-Marie Launay and Benoit Schneider.

15 Tomás Barrio, Alicia Otero, Hasier Eraña, Jorge M. Charco, Belén Marín, Ezequiel González-Miranda, Qhinzhong Kong , Juan M. Torres, Olivier Andréoletti, Juan J. Badiola, Rosa Bolea and Joaquín Castilla.

16 Nuria López, Sonia Veiga and Jesús R. Requena.