Prion Structure and Biology

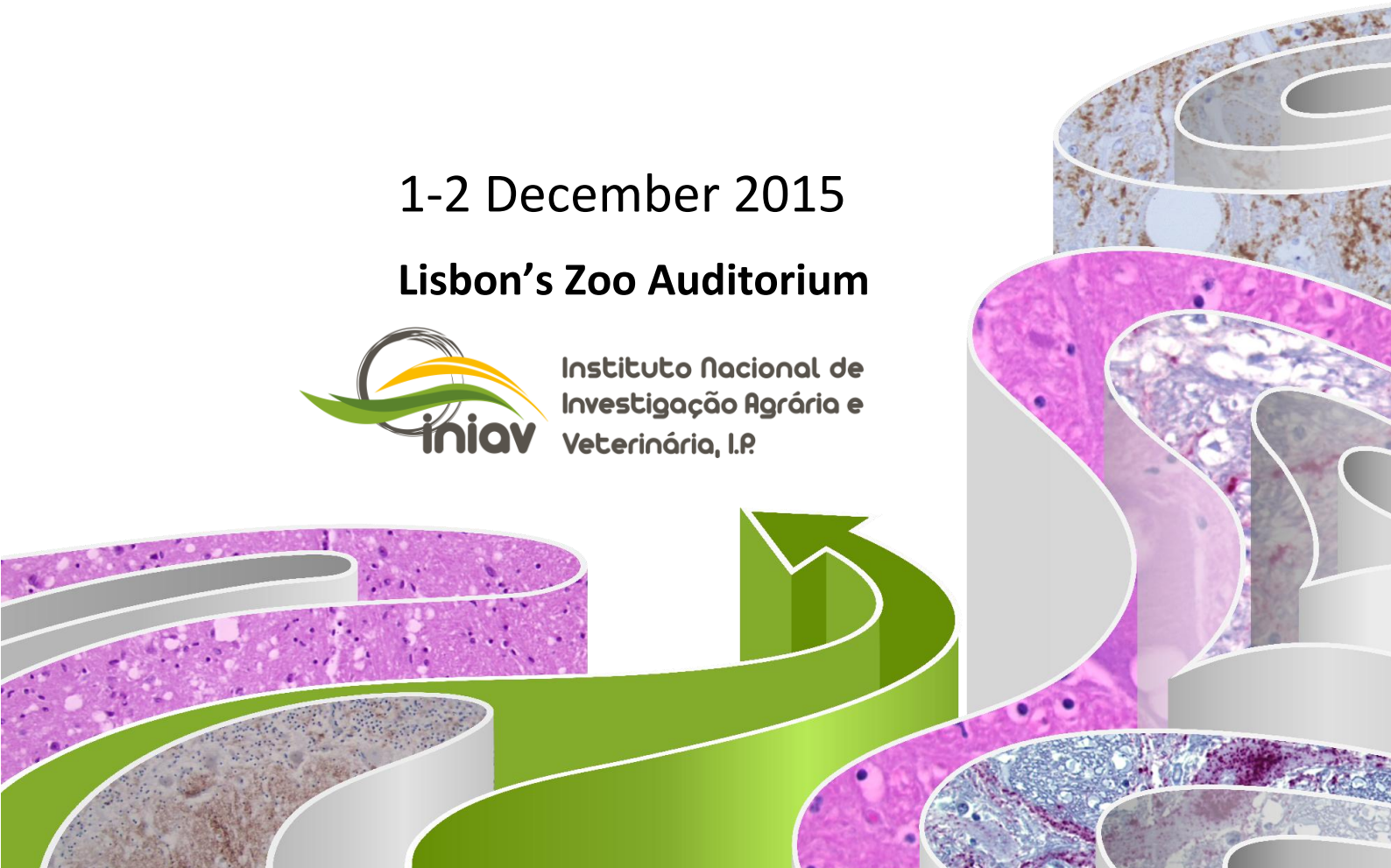
Prion Diseases in Animals

Prion Diseases and Prion-like Diseases in Humans

1-2 December 2015

Lisbon's Zoo Auditorium

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



4th Iberian Congress on Prions

ABSTRACT BOOK

SCIENTIFIC COMMITTEE

- J. R. Requena (Universidad de Santiago de Compostela);
- J. Castilla (CIC-Biogune, Bilbau);
- I. Ferrer (Bellvitage Hospital, Barcelona);
- M. Pumarola (Universitat Autònoma de Barcelona);
- J. Badiola (Universidad de Zaragoza);
- J.M. Torres (CISA, INIA, Madrid);
- T. Mayoral (Ministerio de Agricultura, Alimentación y Medio Ambiente de Espana, Madrid)
- E. Melo (Universidade do Algarve);
- I. Baldeiras (Universidade de Coimbra);
- C. Oliveira (Universidade Coimbra);
- L. Orge (INIAV, Lisboa).

LOCAL ORGANIZING COMMITTEE

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SCIENTIFIC PROGRAM

4th Iberian Congress on Prions

Time	Tuesday 1st December	Time	Wednesday 2nd December
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9:45	**Welcome**	10:00-11:00	**Chairs: J. Badiola and L. Orge** **JM.TOIVONEN** *Analysis of candidate microRNA biomarkers in classical scrapie sheep* **Short Oral Communications** **R. NONNO** *A single amino acid polymorphism in the bank vole prion protein determines strain fidelity upon experimental transmission of L-type bovine spongiform encephalopathy* **JC. ESPINOSA** *Comparison of the transmission features of several prion strains in a transgenic mouse model and in its natural counterpart*
Prion Structure and Biology **10:00-11:00**	**Chair: E. Melo** **Invited Speaker: HOLGER WILLE** ***The infectious prion: A four-rung beta-solenoid structure, however you look at it***		
11:00-11:30	Coffee Break and Poster Discussion	11:00-11:30	Coffee Break and Poster Discussion
11:30-13:00	**Chairs: J. Requena and E. Melo** Oral Communications **J.CASTILLA** *Infectious recombinant prions: In vitro generation and propagation of different strains* **AM.SEVILLANO** *The architecture of recombinants prions is similar to that of brain-derived prions: insight from limited proteolysis* **SR. ELEZGARAI** *Employing dynamic mass redistribution to identify pharmacological chaperones for the cellular prion protein*	11:30-12:00	**Short Oral Communications** **I.GONZALO** *Diversity of PrPd immunorreactivity pattern in atypical Spanish scrapie cases* **C.MACHADO** *Portuguese retrospective BSE typing*
		Prion Diseases and Prion-Like diseases in humans **12: 00-13:00**	**Chair: C. Oliveira** **Invited Speaker: TIAGO OUTEIRO** **From the baker to the bedside: unraveling the molecular mechanisms of Parkinson's disease**
13:00-14:30	Lunch	13:00-14:30	Lunch
14:30-16:00	**Chairs: J. Castilla and J. Requena** **Short Oral Communications** **E.BIASINI** *A cationic tetrapyrrole is a functional inhibitor of the cellular prion protein* **E.MELO** *Getting proteins more compact prevents protein amyloidosis* **S.VENTURA** *Identification of prion-like proteins in proteomes* **JA.MACEDO** *Membrane-enriched proteomic changes associated with prion protein expression during neural differentiation and in neuroblastoma cells*	14:30-16:00	**Chairs: J.Castilla and I. Baldeiras** **L. PIRISINU** *The brains of patients with Gerstmann-Sträussler-Scheinker disease (GSS) harbor transmissible prions* **F.LLORENS** *Pathological and molecular features in Fatal Familial Insomnia: new insights* **W. TAHIR** *Cross talk on the role of PARK7 (DJ-1) in pathophysiology of sporadic Creutzfeldt-Jakob diseases (CJD) in cerebellum in MM1 and VV2 subtypes*
16:00-16:30	Coffee Break and Poster Discussion	16:00-16:30	Coffee Break and Poster Discussion
Prion Diseases in Animals 16:30-18:30	**Chairs: T Mayoral and C. Machado** **R.BUDJOSO** *Faithful replication of mammalian prions in Drosophila* **O.ANDRÉOLETTI** Mono-nucleated blood cell populations display different abilities to transmit prion disease by the transfusion route **S.GILCH** *A polymorphism in the hydrophobic core region of deer PrP modulates biochemical and biological properties of chronic* wasting disease prions **Short oral communications** **A.OTERO** *Effects of N158D canine substitution on prion infected mice* **N. FERNÁNDEZ-BORGES** A comprehensive study of the potential resistance of the canidae family to prion infection **PL.ACUTIS** Prospects for applying breeding for scrapie resistance in goats: the current situation in Italy	**Prion Diseases and Prion-Like diseases in humans** 16:30-18:00	**Chairs: J.M Torres and R. Carvalho** **Short Oral Communications** **B. ANSOLEAGA** *Deregulation of mitochondrial metabolism, protein synthesis and purine metabolism in Creutzfeldt-Jakob disease* **MC. GUERRERO** *Taupatía con rasgos de degeneración corticobasal en la enfermedad de Creutzfeldt-Jakob: perfil neuropatológico y clínico en una serie de casos de banco de cerebros* ***M. GARCÉS*** *Morphological approach to glial involvement in prion and a prion-like diseases* **MJ. LEITÃO** *Novel assay for detection of CSF 14-3-3γ levels increases sCJD diagnosis accuracy*
20:30	Congress Dinner		

PRION STRUCTURE AND BIOLOGY

ABSTRACTS

PLENARY LECTURE- Holger Wille

THE INFECTIOUS PRION: A FOUR-RUNG BETA-SOLENOID STRUCTURE, HOWEVER YOU LOOK AT IT.

Wille H [1] and Requena JR [2]

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The structure of the infectious prion protein (PrP^{Sc}), which is responsible for Creutzfeldt-Jakob disease in humans and bovine spongiform encephalopathy, has escaped all attempts at elucidation due to its insolubility and propensity to aggregate. PrP^{Sc} and its N-terminally truncated variant, PrP 27-30, aggregate into amorphous aggregates, 2D crystals, and amyloid fibrils. The structure of these infectious conformers is essential to understanding prion replication and the development of structure-based therapeutic interventions. We used the repetitive organization inherent to PrP^{Sc} and PrP 27-30 amyloid fibrils to analyze their structure via electron cryomicroscopy and X-ray fiber diffraction. Together, the data define a four-rung β-solenoid structure as the key architecture for infectious mammalian prions. Furthermore, the data refute all previous models for the structure of PrP^{Sc} and allow to formulate a molecular mechanism for the replication of prions. Knowledge of the prion structure provides important insights into the self-propagation mechanisms of protein misfolding that underlie more common neurodegenerative diseases such as Alzheimer's and Parkinson's disease.

SESSION OF ORAL COMMUNICATIONS

INFECTIOUS RECOMBINANT PRIONS: *IN VITRO* GENERATION AND PROPAGATION OF DIFFERENT STRAINS

Castilla J[1, 2], Di Bari MA[3], Sánchez-Martín MA[4], Eraña H[1], Vidal E[5], Sevillano, AM[6], Venegas V[1], Elezgarai SR[1], Gil D[1], Pirisinu, L[3], Moreno J[1], Vázquez-Fernández E[6], Harrathi C[1], Parra B[7], Agostino CD[3], Espinosa JC[8], Surewicz W[9], Torres JM[8], Mayoral T[7], Agrimi U[3], Requena JR[6], Nonno R[3] and Fernández-Borges N[1]

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Prion diseases are a group of fatal neurodegenerative diseases that affect humans and animals and whose main characteristic is its infectious nature. PrP^{Sc}, a misfolded variant of the endogenous PrP^{C}, is the solely pathogenic agent. The infectivity of the misfolded protein was amplified/propagated *in vitro* since a decade ago using Protein Misfolding Cyclic Amplification (PMCA), a technology that has had an enormous impact in the prion field[1]. Recently, a version of PMCA using recombinant PrP (rec-PrP) as substrate (rec-PMCA) has been developed to generate highly PK resistant PrP (rec-PrP^{res}). The infectivity showed by a diversity of rec-PrP^{res} generated *in vitro* by different groups using a variety of co-factors and modified procedures was also diverse. These results confirm: i) the GPI and glycosylation components are not necessary in enciphering an infectious conformation and ii) rec-PrP^{res} can be also structured in the form of different recombinant prion strains with robust *in vitro* self-replicating abilities but dissimilar infectious features *in vivo*.

Our study has been focused on understanding the infectivity and the effect of different cofactors of recombinant prions generated using the polymorphic variant of the bank vole PrP (109I). This model was used as the shortest incubation time model for prion diseases[2] and because of its outstanding susceptibility to propagate most of the existing prion strains from different species[3].

This study shows the *in vitro* generation of infectious recombinant bank vole prions and how cofactors influence over the propagation of certain prion strains with specific infectious features.

[1]PMCA. A Decade of In Vitro Prion Replication. Natalia Fernández-Borges and Joaquín Castilla. Current Chemical Biology. 2010.
[2]Chronic Wasting Disease in Bank Voles: Characterisation of the Shortest Incubation Time Model for Prion Diseases. Di Bari MA, Nonno R, Castilla J, D'Agostino C, Pirisinu L, Riccardi G, Conte M, Richt J, Kunkle R, Langeveld J, Vaccari G,Agrimi U. PLoS Pathog. 2013.
[3]Evidence that bank vole PrP is a universal acceptor for prions. Watts JC, Giles K, Patel S, Oehler A, DeArmond SJ, Prusiner SB. PLoS Pathog. 2014.

THE ARCHITECTURE OF RECOMBINANTS PRIONS IS SIMILAR TO THAT OF BRAIN-DERIVED PRIONS: INSIGHT FROM LIMITED PROTEOLYSIS

Sevillano AM[1], Fernández-Borges N[2], Younas N[1,3], Bravo S[4], Vázquez-Fernández E[5], Elezgarai SR[2], Rosa I[1], Eraña H[2], Vidal E[6], Nonno R[7], Castilla J[2,8], Requena JR[1]

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Substantial evidence suggests that PrP^{Sc} is a 4-rung β-solenoid, and that individual PrP^{Sc} subunits stack to form amyloid fibers. We recently used limited proteolysis to map the β-strands and connecting loops that conform the PrP^{Sc} solenoid. Using high resolution SDS-PAGE followed by epitope mapping, and mass spectrometry, we identified positions ~117/119, 133-134, 152-153, 141, 162, 169 and 179 (murine numbering) as Proteinase K (PK) cleavage sites in PrP^{Sc}. Such sites define loops and/or borders of β-strands, and are helping us define the threading of the β-solenoid.

We have now extended this approach to recombinant PrP^{Sc} ($recPrP^{Sc}$). We apply the term $recPrP^{Sc}$ to *bona fide* recombinant prions prepared by PMCA, exhibiting infectious properties with attack rates of ~100%.

Limited proteolysis of a variety of mouse and bank vole $recPrP^{Sc}$ species, prepared under a variety of slightly different conditions, yields N-terminally truncated PK-resistant fragments similar to those seen in brain-derived PrP^{Sc}. Along with these, doubly N- and C-terminally truncated fragments, in particular ~89/97-152, were detected; similar fragments are characteristic of atypical strains of brain-derived PrP^{Sc}. Resistance to PK of $recPrP^{Sc}$ was lower than that of "classic" PrP^{Sc} and more akin to that of atypical PrP^{Sc} strains. These results suggest that the architecture of $recPrP^{Sc}$ is similar to that of brain-derived PrP^{Sc}. The presence of a mixture of PK-resistant fragments that are characteristic of both "classic" and "atypical" PrP^{Sc} suggests nuances in threading that are specific of $recPrP^{Sc}$, resulting in biochemical properties that are somewhat intermediate between these two PrP^{Sc} types.

Recombinant PrP^{Sc} offers exciting opportunities for structural studies not possible to date with brain-derived PrP^{Sc}.

EMPLOYING DYNAMIC MASS REDISTRIBUTION TO IDENTIFY PHARMACOLOGICAL CHAPERONES FOR THE CELLULAR PRION PROTEIN

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The key pathogenic event underlying all forms of prion diseases is the conversion of the cellular prion protein (PrP^C) into an aggregated form (PrP^{Sc}) that self-propagates by imposing its abnormal conformation onto PrP^C molecules. Previous attempts to identify anti-prion compounds were aimed to reduce the load of PrP^{Sc} aggregates by decreasing their stability or increasing their clearance. Some of these compounds showed potent activity in vitro or in cultured cells, but little or no efficacy in vivo. Multiple pieces of evidence support the notion that PrP^C loses its native fold in the initial steps of the aggregation process. This concept provides a rationale for tackling PrP^C aggregation by stabilizing the monomeric protein precursors, instead of disrupting pre-formed PrP^{Sc} species. The underlying idea is to block aggregation by increasing the Gibbs free energy barrier (ΔG) required for the initial misfolding events. This goal could be achieved with small, high affinity ligands of PrP^C, capable of acting as pharmacological chaperones. In order to identify such compounds, we setup a novel screening method based on Dynamic Mass Redistribution (DMR), a label-free, fully automated biophysical technique performed on 384-well microplates, and capable of detecting molecular interactions at the equilibrium. First, we established optimal buffer composition and efficient immobilization conditions for mouse or human recombinant PrP. We then tested the interaction of a small set of previously characterized compounds to PrP^C. Our analyses confirmed, refined or disputed previous reports, by defining accurate binding constants for each molecule. These results demonstrate that DMR is a reliable platform for the identification of novel PrP^C ligands, providing a unique opportunity for future High Throughput Screening (HTS) campaigns.

SHORT ORAL COMMUNICATIONS

A CATIONIC TETRAPYRROLE IS A FUNCTIONAL INHIBITOR OF THE CELLULAR PRION PROTEIN

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Prion diseases are rare neurodegenerative conditions associated with the conformational conversion of the cellular prion protein (PrP^{C}) into PrP^{Sc}, a self-replicating isoform (prion) that accumulates in the central nervous system of affected individuals. The structure of PrP^{Sc} is poorly defined, and likely to be heterogeneous, as suggested by the existence of different prion strains. The latter represents a relevant problem for therapy in prion diseases, as some potent anti-prion compounds have shown strain-specificity. Designing therapeutics that target PrP^{C} may provide an opportunity to overcome these problems. In fact, PrP^{C} ligands may theoretically inhibit the replication of multiple prion strains, by acting on the common substrate of any prion-replication reaction.

Here, we describe the characterization of the biological properties of a cationic tetrapyrrole [Fe(III)-TMPyP], which was previously showed to bind PrP^{C}, and inhibit the replication of a mouse prion strain. We report that the compound is active against multiple prion strains in vitro and in cells. Interestingly, we also find that Fe(III)-TMPyP inhibits several PrP^{C}-related toxic activities, including the channel-forming ability of a PrP mutant, and the PrP^{C}-dependent synaptotoxicity of amyloid-β (Aβ) oligomers, which are associated with Alzheimer's Disease. These results demonstrate that molecules binding to PrP^{C} may produce a dual effect of blocking prion replication and inhibiting PrP^{C}-mediated toxicity.

GETTING PROTEINS MORE COMPACT PREVENTS PROTEIN AMYLOIDOSIS

Estrela N[1], Lopes C[1], Macedo J A[1], Tavares E [1], Franquelim H G[2], Christiansen G[3], Otzen D E[4] and Melo E P[1]

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The importance of protein folding acquires special relevance in the so-called protein misfolding diseases, such as Alzheimer and prion diseases. Some of these diseases are characterized by the accumulation of aggregated protein forming amyloid fibrils in tissues.

Using the small ribosomal protein S6 as a simple model we have observed that osmolytes such as sucrose delay the formation of amyloid fibrils leading to softer and less-shaped defined fibrils[1]. Osmolytes do not change the folding pathway of S6 but they can promote the compaction of protein conformational states, especially of the unfolded state[2,3] but also of the folded state[4]. Indeed, the delay in S6 amyloidosis by sucrose results from the compaction of the slightly expanded tertiary structure of the native state. Interestingly, this compaction extends to almost all the S6 tertiary structure but hardly affects the secondary structure. Conclusions drawn from the S6 protein can be extrapolated to the prion protein.

[1] Estrela et al., 2015, Proteins, 83 : 2039-2051 ; [2]Chen et al., 2005, J. Mol. Biol. 351, 402-416; [3]Baptista et al., 2008, Biopolymers 89, 538-547; [4]Chen et al., 2006, Biochemistry 45, 2189-2199

IDENTIFICATION OF PRION-LIKE PROTEINS IN PROTEOMES

Ventura S

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Despite major efforts devoted to understanding the phenomenon of prion transmissibility, it is still poorly understood how this property is encoded in the amino acid sequence. In recent years, experimental data on yeast prion domains allow to start at least partially decrypting the sequence requirements of prion formation. These experiments illustrate the need for intrinsically disordered sequence regions enriched with a particularly high proportion of glutamine and asparagine. Bioinformatic analysis suggests that these regions strike a balance between sufficient amyloid nucleation propensity on the one hand and disorder on the other, which ensures availability of the amyloid prone regions but entropically prevents unwanted nucleation and facilitates brittleness required for propagation. This provides a basis for the accurate identification and evaluation of prion candidate sequences in proteomes in the context of a unified framework for amyloid formation and prion propagation[1-3].

1. Sabate, R., Rousseau, F., Schymkowitz, J., Batlle, C. & Ventura, S. Amyloids or prions? That is the question. Prion 9, 200-206 (2015).
2. Zambrano, R., et al. PrionW: a server to identify proteins containing glutamine/asparagine rich prion-like domains and their amyloid cores. Nucleic Acids Res. 43, W331-337 (2015).
3. Sabate, R., Rousseau, F., Schymkowitz, J. & Ventura, S. What makes a protein sequence a prion? PLoS Comput. Biol. 11, e1004013 (2015).

MEMBRANE-ENRICHED PROTEOMIC CHANGES ASSOCIATED WITH PRION PROTEIN EXPRESSION DURING NEURAL DIFFERENTIATION AND IN NEUROBLASTOMA CELLS

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Neurodegenerative diseases are often associated with misfolding and deposition of specific proteins in the central nervous system. Proteomic analysis has emerged as a powerful technology to decipher biological and pathophysiological processes and identify protein biomarkers indicative of disease.

In our study, we intended to analyze protein interactions during neural development and concomitantly the role of the prion protein (PrP) during the process. We focused on the membrane-enriched proteome of differentiating embryonic stem (ES) cells, into neural precursors (NPs), and neuroblastoma cells by means of pre-fractionation and two-dimensional electrophoresis. Enriched scrapie PrP samples from PK1 cells, a prion-susceptible subline of neuroblastoma N2a cells, infected with RML and 22L mouse-infected brain homogenates were compared to cells with no brain and non-infected mouse brain homogenates.

The proteome analysis revealed 158 spots with significantly different expression during neural differentiation, from which 25 proteins were identified by mass spectrometry. Currently, we are trying to verify experimentally the prion infectivity of NPs derived from ES cells. The proteomic approach did not reveal any significant differences between infected PK1 and control cells. However, proteomic expression differed between PK1 and PK1-derived PrP knockdown (KD) cells. The identification of differential proteins between PK1 and KD cells is undergoing and hopefully it will allow gaining insight into the PrP function and possible interacting proteins in neurogenesis and disease.

PRION ANIMAL DISEASES

ABSTRACTS

PLENARY LECTURE- Timm Konold

CLINICAL DISEASE IN TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES OF RUMINANTS

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Scrapie, chronic wasting disease and bovine spongiform encephalopathy are naturally occurring transmissible spongiform encephalopathies (TSEs) in ruminants, which are characterized by accumulation of an abnormal form of the cellular prion protein. Diagnosis of the disease is usually done post mortem by examination of the brain for disease-associated prion protein, and – in the absence of a reliable test in living animals – suspicion of disease is still based on the presence of clinical signs associated with a slowly progressive neurological disease, which can vary between species and TSE strains.

Most research in ruminants has focused on the pathogenesis as well as the pathological, molecular and biological phenotype of naturally occurring and experimentally induced TSEs. Whilst the clinical presentation of naturally occurring TSEs in ruminants has been well documented to aid in the reporting of clinical suspects, little is known about the relationship between clinical signs and neuropathological changes in diseased animals. Initially, clinical signs were attributed to spongiform changes in the brain but this has been questioned following recent research. Similarly, there is little evidence that accumulation of disease-associated prion protein in specific neuroanatomical areas of the brain is associated with corresponding clinical or electrophysiological changes in ruminants. Although these neuropathological markers are used for statutory disease confirmation of TSEs in ruminants they do not appear to be responsible for clinical disease. The question remains whether a prion disease can exist in clinically affected ruminants that is not recognized by those postmortem tests that rely on these markers.

SESSION OF ORAL COMMUNICATIONS

FAITHFUL REPLICATION OF MAMMALIAN PRIONS IN *DROSOPHILA*

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Prion diseases are fatal neurodegenerative conditions that affect humans and a variety of other vertebrate animal species. These diseases, which include scrapie of sheep, bovine spongiform encephalopathy (BSE) of cattle and Creutzfeldt–Jakob disease (CJD) of humans are associated with the misfolding of the normal host protein PrPC into a disease-specific conformer PrPSc. Prion diseases are transmissible between individuals of the same or different species. This poses a significant risk to public health through the potential for zoonotic transmission of prions as evidenced by the BSE epizootic in UK cattle and subsequent emergence of variant CJD in humans. Consequently, much attention has focused on understanding the biology of infectious prions in tissues and body fluids, including blood, from hosts with prion disease.

The only reliable method to detect prion infectivity is by bioassay in an appropriate indicator species. The current bioassays are far from ideal and none can be used to verify the infectious prion potential of large numbers of biological samples in a short period of time. To address this, we have developed PrP transgenic *Drosophila* for use as a tractable bioassay for prion-infectivity in tissues and body fluids from prion-diseased hosts.

We have shown that prion-exposed PrP transgenic *Drosophila* develop a neurotoxic phenotype evidenced by accelerated decline in locomotor ability. This prion-induced phenotype in *Drosophila* is accompanied by accumulation of Proteinase K (PK)-resistant PrPSc and is transmissible to PrP transgenic flies and mice. These are hallmark features of prion replication and indicate that PrP transgenic *Drosophila* can detect mammalian prions. The relative ease of transgenesis in the fly suggests that different genotypes of PrP transgenic *Drosophila* could readily be generated in order to assess prion infectivity from various species. In this context, PrP transgenic *Drosophila* are a novel animal model to investigate the biology of mammalian prion infectivity.

MONO-NUCLEATED BLOOD CELL POPULATIONS DISPLAY DIFFERENT ABILITIES TO TRANSMIT PRION DISEASE BY THE TRANSFUSION ROUTE

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Previous experiments carried out in a sheep scrapie model demonstrated that the transfusion of 200µL has an apparent 100% efficacy for disease transmission. They also indicate that, despite their apparent low infectious titer, the intravenous administration of white blood cells (WBC) result in an efficient disease transmission.

In this study, using the same TSE animal model, we aimed at determining the minimal number of white blood cells and the specific abilities of mono-nucleated cell populations to transmit scrapie by the transfusion route.

Our result confirmed that the transfusion of 100µL but not 10µL of fresh whole blood collected in asymptomatic but scrapie infected sheep donors can transmit the disease. They also show that the intravenous administration of 10^5 WBCs are sufficient to cause scrapie in recipient sheep. Cell-sorted CD45R+ (predominantly B lymphocytes), CD4+/CD8+ (T lymphocytes) and CD14+ (monocytes/macrophages) blood sub-populations displayed comparable infectious titres by bioassays in ovine PrP transgenic mice. However while the intravenous administration of 10^6 CD45+ or CD4/8+ living cells were able to transmit the disease, similar number of CD14+ failed to infect the recipients.

These data support the contention that mononucleated blood cells' populations display different risk to transmit TSE by transfusion route. They also represent an important input for the blood borne TSE risk transmission risk assessment of and for refining the target performance of leuko-reduction processes that are currently applied to mitigate the TSE transmission risk in transfusion medicine.

A POLYMORPHISM IN THE HYDROPHOBIC CORE REGION OF DEER PRP MODULATES BIOCHEMICAL AND BIOLOGICAL PROPERTIES OF CHRONIC WASTING DISEASE PRIONS

Hannaoui S[1], Law S[2], Cheng Y[1], Bollinger T[3], McKenzie D[4], Czub S[5], Gilch S[1]

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Chronic wasting disease (CWD) is a prion disease of North American cervids which affects wild and captive elk, deer and moose. Contrary to most other prion diseases, infectivity is not restricted to the brain, but also detectable in peripheral tissues and bodily fluids, such as muscle, blood, saliva, urine and feces. Environmental prion contamination presumably contributes to the efficient lateral spread of the disease within and between cervid species. Zoonotic transmission has not been demonstrated, although there is evidence suggesting that CWD prions might cross the species barrier to humans upon passage and strain adaptation. Although the PrP primary structure is highly conserved between cervids, the disease phenotype can be modulated by species-specific polymorphisms in the prion protein gene.

We have characterized CWD isolates from elk, mule deer and white-tailed deer. In addition to varieties between CWD prions from these species, we found remarkably different properties even between isolates from individual animals of the same species. In white-tailed deer, conformational stability of two isolates was different, and upon sequencing we identified one animal harboring an allele with an A116G exchange, whereas the second animal harbored the wild-type (wt) PrP genotype. The 116AG prions displayed lower conformational stability, lower seeding activity in RT-QuIC, and lower infectivity in cultured cerebellar granular neurons derived from transgenic mice overexpressing cervid PrP. Notably, upon intracerebral injection of transgenic mice with 116AG CWD prions, incubation time of disease and duration of clinical phase were significantly prolonged compared to wt CWD prions.

In summary, polymorphisms in cervid PrP can significantly alter prion conformation and development of disease. Conformation is an important criterion when assessing transmission barrier, and conformational variants can target a different host range. Therefore, thorough analysis of CWD isolates and re-assessment of species barriers is important in order to fully exclude zoonotic potential of CWD.

SHORT ORAL COMMUNICATIONS

EFFECTS OF N158D CANINE SUBSTITUTION ON PRION INFECTED MICE

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Transmissible spongiform encephalopathies (TSEs) or prion diseases are a group of neurodegenerative processes of animals and humans. The conversion of a physiologically encoded mammalian protein (PrP^{C}) in an abnormally folded protein known as PrP^{Sc} is involved in the pathogenesis of these diseases. Molecular mechanisms implicated in this conversion are not completely known. Although a wide variety of species have developed a prion disease, natural or experimentally, some species such as the dog have been reported to be resistant or lowly susceptible to contract a TSE. In our study we used transgenic heterozygous mice expressing a unique acidic substitution of dog PrP^{C}, the change of asparagine to aspartic acid (Tga20xN158D mice). Animals were inoculated with four mouse-adapted prion strains: 22L, RML, 301C and ME7. Clinical signs, survival times, spongiform lesions and PrP^{Sc} deposits of Tga20xN158D mice were compared with those observed in Tga20xKO and Tga20 mice inoculated with the same strains. Even though all animals developed disease and they showed similar histopathologic features and PrP^{Sc} deposition profiles when they were inoculated with the same strain, Tga20xN158D mice showed significantly longer survival times than those observed in Tga20 and Tga20xKO mice. This finding suggests that this dog aminoacidic substitution induces an inhibition over PrP^{C} conversion to PrP^{Sc} isoform.

A COMPREHENSIVE STUDY OF THE POTENTIAL RESISTANCE OF THE CANIDAE FAMILY TO PRION INFECTION

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Prion resistant species have been of special interest in the field because of their potential in deciphering essential determinants for infectivity. Previously, our group has been involved in the development of different *in vitro* and *in vivo* models to assess the susceptibility of species that as *Leporidae* and *Equidae* families were erroneously considered resistant to prion infection. Here, we show a similar *in vitro* and *in vivo* approach developed to understand the unprecedented low prion susceptibility of the family *Canidae*.

Initially, studies based on the amino acid sequence of the *Canidae* PrP have allowed us to identify unique key amino acids whose characteristics could orchestrate this high resistance. Cell- and brain-based PMCA studies among others have been performed highlighting the relevance of certain amino acids. An *in vivo* confirmation was performed through the generation of transgenic mouse models carrying the substitutions of interest. A complete resistance after intracerebral challenge using several prion mouse strains was observed.

These findings are of particular interest also to decipher those negative dominant determinants that might be used on new therapeutic approaches against prion diseases.

PROSPECTS FOR APPLYING BREEDING FOR SCRAPIE RESISTANCE IN GOATS: THE CURRENT SITUATION IN ITALY

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The selection of the resistance-associated PrP allele 222K could be an effective tool to control classical scrapie in goats, both in the general population and as an eradication measure in outbreaks, through selective culling. In Italy, through an European project (GOAT-TSE-FREE), the project RF2010-2318525 and an initiative coordinated by the Italian Ministry of Health, actions were put in place in order to: 1) involve farmers, sensitizing them to breed 222K goats; 2) genotype a large number of goats to locate resistant animals; 3) manage scrapie outbreaks postponing any culling for 3 years, to increase 222K animals in the meantime. The general aim was to prepare the basis for a future breeding program. Meetings with farmers are being organized at regional level, informing about 222K resistance and offering the opportunity of having their animals genotyped for free. So doing, about 5000 goats have been genotyped, belonging to 13 different breeds. Percentages of resistant animals ranged from 2.5% to 16% in Northern breeds and from 16% to 18% in Southern breeds. In 2015 four goat scrapie outbreaks were managed as above mentioned, in which resistant carriers were found at percentages from 10% to 30%. All the results of genotyping have been transferred to the farmers. A national database was created with the records of all the genotyped animals allowing the trace back of resistant animals. This database has been used to find resistant bucks to be introduced in an outbreak, for repopulation. All the activities done so far showed that farmers are interested in breeding resistant goats, but that a breeding program will be more difficult than for sheep, given the low frequency of 222K carriers. Our strategy will provide evidence on the opportunity of replacing the stamping out of goats outbreaks with selective culling and will facilitate breeding for resistance initiatives.

ANALYSIS OF CANDIDATE MICRORNA BIOMARKERS IN CLASSICAL SCRAPIE SHEEP

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Ovine scrapie is a prototypic prion disease. Although scrapie is not traditionally considered zoonotic, recent data suggest that i) scrapie transmission to humanized mice is similar to classical BSE and ii) direct transmission to primates occur albeit with relatively longer incubation periods compared with BSE. Scrapie diagnosis is currently based on clinical symptoms and post-mortem analysis of PrP^{Sc} in the central nervous system (CNS). The identification of easily accesible, rapid and economical early biomarkers could facilitate the isolation of preclinical animals and eradication of disease. Dysregulation of microRNAs (miRNAs), small non-coding RNA molecules involved in post-transcriptional regulation of gene expression, is frequently found in CNS and body fluids in many neurodegenerative diseases (Alzheimer's, Parkinson's, ALS etc). Some miRNAs have been reported to display altered expression in the CNS of mice infected with mouse-adapted scrapie, in post-mortem samples of human TSEs and in exosomal vesicles released by prion-infected cells. However, whether circulating miRNA alterations occur in TSEs in vivo is currently unknown. Here, candidate miRNAs were analysed in cervical spinal cord (medulla cervical, MEC) and in circulating blood plasma of scrapie sheep. Only one of these (miR-21-5p) displayed a close-to-significanct increase in the scrapie MEC vs. healthy controls. However, a correlation with histopathological lesions was observed for three others (miR-342-3p, miR-132 and miR-128-3p). In circulating blood plasma, significant increase was found for miR-21-5p and miR-342-3p. To our knowledge, this is the first description of circulating miRNA alterations in any live animal suffering from TSE. Although genomic level studies are warranted to investigate the true depth of miRNA alterations in the circulation of TSE animals and patients, especially those present in pre-clinical phase, our work demonstrates the potential feasibility of miRNAs as TSE biomarkers in the future.

SHORT ORAL COMMUNICATIONS

A SINGLE AMINO ACID POLYMORPHISM IN THE BANK VOLE PRION PROTEIN DETERMINES STRAIN FIDELITY UPON EXPERIMENTAL TRANSMISSION OF L-TYPE BOVINE SPONGIFORM ENCEPHALOPATHY

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Bank vole is a rodent species highly susceptible to several human and animal prion strains. We have previously shown that in most, but not all, prion transmission experiments the resulting vole-adapted strains reproduce several molecular and pathological features of the inoculated prion diseases. However little is known about the biological properties of vole-adapted strains, i.e. to what extent animal-derived vole-adapted prions preserve the strain properties of the incoming prion.

In this study we investigated the transmission features of L-type bovine spongiform encephalopathy (L-BSE) in two genetic lines of bank voles, carrying either methionine or isoleucine at codon 109 of the prion protein (named Bv109M and Bv109I, respectively). Furthermore, we investigated the strain fidelity of vole-adapted L-BSE by bioassay in tg110 mice, a transgenic mouse line overexpressing bovine prion protein.

L-BSE was transmissible in voles, although transmission in the two vole genetic lines occurred with different efficiency and was accompanied by different PrP^{Sc} features. Transmission in Bv109M gave very long survival time and was accompanied by an upward shift of the molecular weight of PrP^{Sc} compared to the cattle inoculum, suggesting that L-BSE strain might have mutated after transmission in Bv109M. In contrast, transmission in Bv109I gave shorter survival time and was characterised by a PrP^{Sc} type similar to the cattle inoculum, suggesting that the L-BSE strain features might have been preserved. These results were corroborated by bioassay in tg110 mice, where Bv109I-adapted L-BSE, but not Bv109M-adapted L-BSE, proved to have fully preserved L-BSE strain features.

Overall, our findings show that L-BSE strain features were preserved after two passages in Bv109I voles, although PrP in Bv109I has several amino acid mismatches compared to cattle PrP. In contrast, the presence of methionine at codon 109 in vole PrP prevented the faithful replication of L-BSE and caused permanent strain mutation.

COMPARISON OF THE TRANSMISSION FEATURES OF SEVERAL PRION STRAINS IN A TRANSGENIC MOUSE MODEL AND IN ITS NATURAL COUNTERPART

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Bank vole is a rodent species that shows differential susceptibility to a wide range of prions from diverse species. In this work, the transmission features of a panel of diverse prions with distinct origin has been assayed in both bank vole (Met109 variant) or in transgenic mice expressing physiological levels of bank vole PrP^C on a mouse PrP null background (VoPrP-Tg407 mouse line). This work compile the first systematic comparison of the transmission features of a collection of TSE isolates, representing a panel of diverse prion strains, in a transgenic mouse model and in its natural counterpart. The results showed very similar transmission properties in both natural species and transgenic mouse model. Taken together these results demonstrate that the differential susceptibility of bank vole to prion strains observed here and also previously reported can be modelled in transgenic mice expressing vole-PrP, suggesting that this selective susceptibility is only modulated by the vole PrP sequence rather than by other species specific factors. Moreover, our results confirm the pivotal role of the host PrP sequence in prion transmission and that the transmission barrier phenomenon is linked to the prion strain involved.

The main diference between the natural species and transgenic mouse model expressing physiological levels of bank vole PrP^C was observed in bank vole inoculated with BSE-derived prions where a diversity of PrP^{res} profiles in western blot was observed while in the transgenic mouse model a unique PrP^{res} (BSE-like) profile was reported. This difference suggests that genetic factors others than PrP^C sequence can have some background effect in this substrain selection phenomenon.

DIVERSITY OF PRP^D IMMUNORREACTIVITY PATTERN IN ATYPICAL SPANISH SCRAPIE CASES.

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Scrapie is a Transmissible Spongiform Encephalopathy (TSE) in small ruminants comprising two major strains, the classical one and other atypical also known as Nor 98. Atypical Scrapie shows distinctive features and was identified firstly in Norway (Benestad et al 2003) although similar cases have been reported in several European countries.

Since 2001, more than 200 atypical cases have been identified by discriminatory and confirmatory methods in Spain from the implemented TSE Surveillance Program according to the EU rules (R. CE 999/2001).

The aim of this work is to study the distribution of PrP^d by immunohistochemistry in those atypical Scrapie cases where cerebellum fixed-tissue was available. Samples were detected in a first analysis by rapid tests analysis based on two colorimetric ELISA methods (Bio-Rad TeSeE and Herd Check BSE- Scrapie antigen test) and discriminatory assay according to AHVLA Bio-Rad TeSeE-based Hybrid Western blotting. Histology (H&E) and immunohistochemicals (IHQ) techniques were performed using 2G11 (Institute-Pourquier) monoclonal antibody.

According to the of PrP^d immunoreactivity in the cerebellum, PrP^d deposits can be identified in both layers (molecular and granular) showing two main patterns: a granular-type one and a fine punctate-type in a diffuse o focal way. Different combinations of them are observed in each layer: only one type presence (granular or fine-puntacte types), both patterns detected (granular and fine-punctate types) and a non defined pattern involving one negative layer while the other remains positive, with granular or fine-punctate type, either individually or collocated. Moreover, most of cases showed also small deposits of glial type in the white matter, although results show as atypical cases detected in cerebellum, can display a diversity of patterns for the PrP^d immuno-staining.

PORTUGUESE RETROSPECTIVE BSE TYPING

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Since 1994, when the first Portuguese BSE case was diagnosed, until now, a total of 1089 BSE cases were detected, most of them belonging to the risk population of the Active Surveillance Programme. Having reached its peak in 1999, since then, the number has been decreasing until only 1 case in 2012 and 1 in 2014. In February 2014, Portugal was recognized as being of negligible risk for BSE by the OIE Scientific Commission. Following the possibility set in Decision 2013/76/EU and EFSA report of 8 October 2012, Portuguese authorities decided to stop testing bovine healthy slaughter population (only risk population, clinical suspects and compulsory slaughter).

With the growing importance of knowing the type of BSE strain (-C, -H and –L) existing in each country, July 2013 was the startpoint for the compulsory discriminatory testing, using the western immunoblotting method, of all cases detected in order to identify possible atypical ones. Some Member States (Portugal included) started, by its own initiative, this study in bovine samples resulting positive from 2010 (confirmation with both Immunohistochemistry, if possible, and double Immunolabeling Western Blot), with the detection of our first BSE-H in 2012 in a BARB animal (born in 1998). As data before 2010 was scarce, the European Commission launched a retrospective study of BSE positive cases between 2003 and 2010. Portugal has a total of 294 cases within this study.

As confirmation routine in our laboratory and also for this work, the protocol used is the AHVLA Bio-Rad TeSeE-based Hybrid Western Blotting Method, with the reagents from the TeSeE™ Western Blot Kit (BioRad) and P4, from r-Biopharm, as the second mAb. For the *Prnp* analysis, we use the *Wizard® Genomic DNA Purification Kit (Promega)* for DNA extraction, PCR amplification (Choi *et al*., 2012; Sander *et al*., 2004) and Sequencing (Choi *et al.,* 2012) with DNA ABI3730xl.

This work will present the results obtained in this retrospective study.

POSTER SESSION

POSTER 1

EXPERIMENTAL TRANSMISSION OF A SPANISH CLASSICAL SCRAPIE ISOLATE TO A COW

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Scrapie was the first transmissible spongiform encephalopathy (TSE) to be described. Epidemiological evidence suggested that classical bovine spongiform encephalopathy (BSE-C), the most important TSE for public health as responsible for causing the new variant of Creutzfeldt-Jakob disease (vCJD), was derived from the scrapie agent included in the rendering process in cattle feed. Several studies have shown that cattle are susceptible to different classical scrapie isolates when inoculated by the intracerebral route. The resulting bovine scrapie is characterized by clinical presentation and histopathological changes which differ from BSE accompanied by a PrPSc accumulation restricted to the central nervous system (CNS). The present study describes the pathological findings observed in a calf intracerebrally inoculated with a Spanish natural scrapie isolate. Clinical disease was observed 30 months post inoculation. Animal stood motionless with the head lowered or resting against objects and did not over-react to external stimuli. No significant spongiform changes were shown by histopathology. The immunohistochemical study using monoclonal antibody (mAb) L42, revealed a PrPSc distribution throughout the CNS. Moreover, PrPSc was detected in peripheral nerves, lymphoid tissues, skeletal muscle, gastrointestinal tract, pancreas and adrenal gland. By using Protein Misfolding Cyclic Amplification method, PrPSc was detected on spleen after tow 48 hours consecutive rounds on Tg338 brain homogenate as substrate. To our knowledge, this is the first report of PrPSc detection in tissues more than the CNS in experimental transmission of classical scrapie into cattle. The Western Blotting analysis by using mAb P4 was not able to detect bovine-scrapie signal, however, the molecular signature detected by mAb Sha31 differed from BSE-C and original scrapie isolate. The study showed that this isolate of classical scrapie, could be transmitted to cattle by intracerebral inoculation and cause a fatal neurological disorder. However, many features of this experimental disease distinguished it from BSE.

POSTER 2

LONG TERM PERSISTENCE OF PRION INFECTIVITY IN AQUATIC ENVIRONMENTS

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Environment plays a key role on horizontal transmission of prion diseases since prions are extremely resistant to classical inactivation procedures. A previous study in our laboratory *(Maluquer et al., Environmental Research, 2012)* proved the long maintenance of BSE infectivity when prions are incubated in aquatic environments by means of mouse bioassays. No significant reduction in BSE infectivity was observed after incubation in PBS for near one year while a two orders of magnitude reduction was reported after incubation in residual wastewater for the same period.

As a continuation of this experiment, the same samples were incubated in PBS and wastewater for five additional years and the residual BSE infectivity was assessed in bovine PrP transgenic mice. During this long time, BSE infectivity was reduced by three orders of magnitude on wastewater but only by one order of magnitude on PBS. To rule out a possible strain specific effect, the same experimental procedure where applied to sheep scrapie prions, using eight years as the experiment end-point. No significant reduction in scrapie infectivity was observed after wastewater incubation during the first year of incubation. The infectivity data corresponding to eight years incubation in wastewater could not be obtained due to inoculum toxicity, but samples incubated in PBS during eight years showed only a two orders of magnitude reduction in infectivity. In contrast, the dynamics of persistence for PrP^{Res} were different which disappeared progressively during the first year.

The low reductions in prion infectivity obtained in this study, using two different strains, reinforce the idea of a high stability of these agents in aquatic environments and that environmental processes or conventional water treatments with low retention times applied to wastewater and sewage would not have any impact on prion infectivity. These results could have a great repercussion in terms of risk assessment and safety for animals and human populations.

POSTER 3

EVALUATION OF THE CONTRIBUTION TO PATHOLOGICAL PRION PROTEIN FORMATION FROM THE 222K PRP VARIANT IN SCRAPIE POSITIVE GOATS BY A BIOCHEMICAL APPROACH

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Genetic studies have shown that the caprine *PRNP* gene is highly polymorphic. More than 40 polymorphisms have been identified to date, and some of them can modify susceptibility to scrapie. Among these polymorphisms, the presence of lysine (K) at position 222 of goat PrP is associated with resistance to classical scrapie, yet few natural cases of scrapie have been found in Q/K goats and intracerebral experimental transmission was successful in Q/K and K/K goats after long incubation periods. Recent western blot studies performed by different monoclonal antibodies (mAbs) revealed an inhibition by K222 on the binding of mAb F99/97.6.1 to goat PrP. Thus a western blot method, based on the ratio between the signal intensity given by mAbs F99/97.6.1 and SAF84, was developed to distinguish goats exhibiting PrP encoded by the genotypes 222Q/Q, 222Q/K and 222K/K. Here we applied this approach to investigate the contribution to PrP^{Sc} formation given by the 222K variant in scrapie positive goats. Western blot analyses were performed on the PrP^{C} or PrP^{Sc} extracted from the brains of negative and scrapie positive goat samples (natural or experimental), bearing different genotypes at position 222. PrP signals were detected by mAbs F99/97.6.1 and SAF84 simultaneously and then quantified. Each sample was analyzed in triplicate. A descriptive statistical analysis was performed. The results obtained from these investigations showed no PrP signals by F99/97.6.1 in any of the 222K/K goat samples. The ratio of the optical densities revealed that the positive 222Q/K goats had a similar reactivity to 222Q/Q samples. Halved values of the ratio were present in negative goats with 222Q/K. The statistical analysis confirmed these differences. The obtained results showed that in heterozygous animals PrP^{Sc} seems to be formed nearly totally by the Q222 variant thus confirming the higher resistance to convertibility of K222 PrP variant

POSTER 4

COMPARATIVE STUDY BETWEEN CAPRINE BSE AND BOVINE BSE IN EXPERIMENTALLY INOCULATED GOATS WITH DIFFERENT MUTATIONS AT CODON 222 OF *PRNP* GENE

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Bovine spongiform encephalopathy (BSE) represented one of the largest food crises in the last decades. The efforts of the European Union have managed to almost eradicate the disease in cattle; however, the BSE agent has demonstrated a high capacity to cross species barriers and other ruminant species may have been affected. Until now, relevant aspects such as pathogenesis, transmission or genetic factors of these possible cases have not been studied in depth. This work compare two experimental studies that attempt to define the importance of different mutations at codon 222 of *PRNP* gene in the distribution of PrP^{Sc} in goats after intracerebral inoculation with BSE. In the first study, nine animals were inoculated with caprine BSE agent; while in the second study, still under development, five animals were inoculated with bovine BSE. In both studies, goats carried the homozygous genotypes Lysine 222KK and Glutamine 222QQ; and in the first study also the heterozygous Glutamine/Lysine 222QK. Most of 222QQ animals from both studies presented clinical signs consistent with BSE after 15 months in case of caprine BSE goats, and after 19±2 months in bovine BSE individuals. 222KK animals inoculated with bovine BSE are still alive without any clinical signs after more than 24 months post inoculation. A comparative study about the severity and distribution of vacuolation was performed. PrP^{Sc} was identified by the technique of Western Blot in the obex of all genotypes, and differences were only detected in terms of signal intensity. Immunohistochemistry was used to evaluate the distribution of PrP^{Sc} in the brain, finding greater accumulation in 222QQ animals. In addition, a wide distribution of PrP^{Sc} was observed throughout the organism, but always associated with lymphoid tissue or peripheral nerve fibers.

POSTER 5

CORRELATION BETWEEN PRION LESIONS AND AUTOPHAGY MARKERS IN THE CENTRAL NERVOUS SYSTEM OF SCRAPIE INFECTED SHEEP

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Autophagy is a dynamic cellular pathway involved in the turnover of proteins, protein complexes, and organelles through lysosomal degradation. The appearance of multi-vesicular bodies and autophagic vacuoles has been reported in several models of prion diseases but their relationship with prion-related lesions has never been investigated. We present here the expression profiles of four autophagy related genes (*ATG5, ATG6, ATG9* and *LC3*) and the protein distribution of ATG5, LC3-I and LC3-II in several areas of the central nervous system of sheep naturally infected with classical scrapie and their age/breed/genotype matched controls. In addition, scrapie-related histopathological lesions (prion deposition, neuronal vacuolation and neuropil spongiosis) were evaluated in the same brain areas and their correlation with autophagy markers was evaluated. All four genes showed variable expression profiles in the different areas. Nevertheless a significant *LC3* downregulation was detected in the medulla oblongata, the most affected area in these diseases. Immunostaining of ATG5, LC3-I and LC3-II did not explain the gene expression levels; on the contrary, upregulation of theses markers was observed in specific neural populations of the medulla oblongata and pons. In addition, an intense LC3-I and LC3-II staining was observed in the Purkinje cells in cerebellum of scrapie animals whereas controls showed none or very low staining signals. A positive Spearman's rho correlation was observed between LC3-I immunostaining and PrP^{Sc} deposition (ρ=0.648, $p<0.05$), neuronal vacuolation (ρ=0.544, $p<0.05$), neuropil spongiosis (ρ =0.694, $p<0.01$) and with its membrane-bound form LC3-II (ρ =0.734 $p<0.01$). The correlation observed between prion lesions and autophagy markers and the intense immunostaining of LC3-I and LC3-II in specific neuronal populations suggests that autophagy plays a neuroprotective role against the toxicity induced by prions.

POSTER 6

NOR98 PRP^{SC} IS UNABLE TO INDUCE BANK VOLE PRP^{C} MISFOLDING BY PMCA

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Despite PMCA was reported to replicate several prion strains, the *in vitro* amplification of TSEs characterized by atypical PrP^{Sc}, with protease-resistant core cleaved at both the N and C-termini, hasn't been reported so far. We recently showed that bank voles carring isoleucine at PrP residue 109 (Bv109I), but those carrying methionine (Bv109M), are susceptible to Nor98 and GSS[1,2], both characterized by atypical PrP^{Sc}, suggesting a central role of host PrP^{C} sequence in the permissiveness to these TSEs. We thus aimed at investigating if this principle operates *in vitro* too, by attempting to replicate *in vitro* Nor98 by PMCA on brain substrates from Bv109M and Bv109I.

In PMCA reactions performed with our standard protocol[3], no amplification was observed in both substrates using either ovine or vole-adapted Nor98 seeds. Although a PrP^{res} signal decrease was occasionally observed in samples subjected to PMCA, further experiments showed that neither continuous sonication up to 5 minutes nor incubation at 90°C affected the stability of Nor98 PrP^{Sc}. Negative results were also obtained by changing length of PMCA cycles, detergents or by adding Teflon beads in PMCA tubes.

This absence of amplification might simply represent a strain feature of Nor98, as different prion strains show different replication efficiencies in PMCA. Alternatively, atypical PrP^{Sc} itself might be unable to induce *in vitro* aggregation of PrP^{C}, supposedly because of peculiar mechanisms of aggregation not supported by PMCA. To investigate this hypothesis we are now testing other TSEs with atypical PrP^{Sc} for their ability to replicate in PMCA.

POSTER 7

COMPARISON OF TWO MURINE TRANSGENIC LINES IN THE CHARACTERIZATION OF SCRAPIE PRION STRAINS FROM ARAGÓN (SPAIN)

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Traditionally, murine bioassays have been used as an effective methodology for characterizing different prion strains responsible for natural transmissible spongiform encephalopathy cases. Distinct prion strains show different phenotypes of the disease following serial passages in mice. These murine models have progressed with the development of transgenic mice lines that bear the PRNP gene of other species, such as sheep in the case of Scrapie, in order to skip the transmission barrier.

In these studies, clinical symptomatology and incubation time are recorded, as well as lesion profile, immunohistochemical profile and, more recently, glycosylation pattern.

This study is conceived as the next step of a previous research, in which two serial passages of 22 isolates from naturally Scrapie-infected sheep were performed using TgShp transgenic mice. The data gathered from these animals suggested some diversities regarding the strain in the nervous and/or lymphoid tissue of sheep coming from several Scrapie outbreaks in Aragón.

In the present bioassay, currently underway, the transgenic murine line Tg338 was used. In the first passage, a high attack rate (>90%) was observed, near to that in TgShp. Incubation periods (IPs) from first passage animals were similar to those reported in TgShp. Moreover, the high PrP^C expression levels of this line (8 to 10-fold that of sheep) are suspected to be involved in the noticeable shortening of the IP that is being observed in the ongoing second passage.

Additionally, nervous tissue samples collected from first passage Tg338 mice are currently being processed in order to obtain the lesion and immunohistochemical profiles and the glycosylation pattern, which will be compared with those from TgShp mice.

POSTER 8

SCRAPIE CASES DIAGNOSED IN POLAND

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Scrapie in small ruminants and bovine spongiform encephalopathy (BSE) in cattle are the most important prion diseases of animals and active surveillance in Member States for both diseases is obligatory.

The aim of the study is to present the epidemiological data on scrapie diagnosed in Poland in years 2002-2015 (until the end of September).

Since 2002 rapid tests were introduced in Polish small ruminants. Brain stem samples are tested at the age of 18 months for healthy slaughtered animals and above 12 months for risk group. In years 2002-2005 the majority of labs used Bio-Rad test. In 2006, Prionics LIA test was introduced and it was used until the end of 2008. Then Idexx test was implemented and since then it is constantly used. All initially reactive samples are sent to NRL for Animal TSEs in Pulawy, for confirmatory diagnosis. The *PRNP* genotypes and alleles are investigated by sequencing in Balice.

Overall 65 cases in sheep were diagnosed of which 34 were found in Polish flocks. Imported cases came from Slovakia (13 cases), Romania (15 cases), Hungary (2 cases) and Spain (1 case). All of Polish cases were Nor98 (atypical scrapie). Majority of Polish scrapie cases were found in fallen stock (85%). Mean age of Polish Nor98 cases was 91 months. The youngest Nor98 native case was diagnosed in 42 months old sheep. The most dominant genotypes of Polish cases of Nor98 were NSP3 (42%) and NSP2 (31%). The presence of phenylalanine at codon 141 does not seem to be a risk factor for Nor98 in Polish sheep, since only 23% of cases had this polymorphism (L/F). All other cases were L/L homozygotes. The incidence of the most susceptible genotypes, classified in NSP5 group, is very low in Poland, which probably hampers the occurence of classical scrapie in small ruminants.

POSTER 9

PROCESSED ANIMAL PROTEINS (PAPs) IN FEED

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In European Union, each Member State must carry out an annual program for monitoring bovine spongiform encephalopathy (BSE). The use of processed animal proteins (PAPs), including meat and bone meal (MBM), in meat producing animals for human consumption is forbidden in the European Union as a consequence of the BSE crisis.

The prohibition of PAPs in feed is the only way to prevent the spreading of transmissible spongiform encephalopathy (TSE) to which the mad cow disease belongs.

INIAV is simultaneously an official control laboratory as well as the Portuguese National Reference Laboratory for animal proteins in feeding stuffs (NRL-AP), ensuring the official control laboratories network, the dissemination of information from European Reference Laboratory, training, quality assistance, organization of national proficiency tests and collaboration with the Competent Authority.

The EU Regulation 56/2013, on TSEs, reforms the strict rules of "Feed Ban" on the use of PAPs from non-ruminants (e.g. pigs and poultry) in feed and allows PAPs derived from non-ruminant animals to be used in aqua feed.

There are several analytical techniques which can be used to detect the presence of animal proteins in feed but the official validated methodologies are described in Regulation (EU) nº 51/2013. The reference microscopic method focus on the presence of bone fragments, but muscle fibers, feathers, cartilages and hairs are targeted as well. It allows to distinguish the presence of constituents derived from terrestrial animals from those derived from fish. Concerning the ban lifting of PAPs from non ruminants in feed for aquaculture and for fur-producing animals, a new method focused on ruminant DNA detection based on real time Polymerase Chain Reaction – real time PCR - was validated, by the EU reference laboratory, and implemented on Portuguese NRL to detect the presence of ruminant's constituents in feedingstuff.

PRION DISEASES AND PRION LIKE DISEASES IN HUMANS

ABSTRACTS

PLENARY LECTURE- Tiago Outeiro

FROM THE BAKER TO THE BEDSIDE: UNRAVELING THE MOLECULAR MECHANISMS OF PARKINSON'S DISEASE

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Aggregation of alpha-synuclein (ASYN) in Lewy bodies and Lewy neurites is the typical pathological hallmark of Parkinson's disease (PD) and other synucleinopathies. Furthermore, mutations in the gene encoding for ASYN are associated with familial and sporadic forms of PD, suggesting this protein plays a central role in the disease. However, the precise contribution of ASYN to neuronal dysfunction and death is unclear. There is intense debate on the nature of the toxic species of ASYN, and little is still known about the molecular determinants of oligomerization and aggregation of ASYN in the cell. Harnessing the power of various model organisms, we are making progress towards the understanding of the basic molecular mechanisms underlying PD and other synucleinopathies. In order to clarify the effects of different posttranslational modifications on the toxicity and aggregation of ASYN, we employ a variety of model systems. Phosphorylation and glycation are emerging as important modifications affecting ASYN aggregation. Altogether, our data shed light into the molecular underpinnings of PD, and open novel perspectives for therapeutic intervention in synucleinopathies.

SESSION OF ORAL COMMUNICATIONS

THE BRAINS OF PATIENTS WITH GERSTMANN-STRÄUSSLER-SCHEINKER DISEASE (GSS) HARBOR TRANSMISSIBLE PRIONS

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Gerstmann-Sträussler-Scheinker disease (GSS) is an inherited neurodegenerative disorder associated with mutations in the prion protein gene. It is characterized by atypical PrP^{Sc} with an internal protease-resistant core (PrP^{res}) of 6-8 kDa. Attempts to transmit GSS in rodents have showed that only the few cases accompanied by 21 kDa PrP^{res} were transmissible, leading to the hypothesis that GSS exclusively associated with the internal PrP^{res} are non-transmissible proteinopathies rather than true prion diseases. As bank vole is considered a universal acceptor for prions, we hypothesized that, if transmissible, GSS prions could propagate and cause neurodegeneration in voles. Indeed, GSS cases with P102L, A117V and F198S mutations were able to induce prion disease in voles, irrespective of the presence of 21 kDa PrP^{res} in the inoculum. The most efficient transmissions were observed with A117V inocula harboring internal PrP^{res} fragments only, which induced clinical disease within 3 months upon primary transmission as well as upon second passage. Notably, all GSS inocula induced the accumulation of internal PrP^{res} fragments, although a P102L case with 21 kDa PrP^{res} led also to the accumulation of 21 kDa PrP^{res} in some recipient voles. Furthermore, it was possible to discriminate between 7 and 8 kDa PrP^{res} fragments in recipient voles, with the latter being induced only by P102L cases.

These findings imply that brains of GSS patients harbor transmissible prions, with features similar to those of classical prions. Finally, the presence of distinct disease phenotypes in inoculated voles is consistent with significant prion strain variation in GSS, which could contribute to their marked clinical and pathological heterogeneity.

PATHOLOGICAL AND MOLECULAR FEATURES IN FATAL FAMILIAL INSOMNIA: NEW INSIGHTS

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Mutations in PRNP are causative of genetic prion diseases the phenotype of which depends on the site of PRNP mutation but also on the polymorphism of codon 129 in PRNP.

Fatal familial insomnia (FFI) is an autosomal dominant prion disease caused by a D178N mutation in *PRNP* in combination with methionine at the codon 129 in the mutated allele of the same gene (D178N-129M haplotype).

Clinically, FFI is principally manifested as sleep disturbances with insomnia, sleep fragmentation, and altered arousal and dreaming; accompanied by autonomic disturbances including increased salivation and sweating, tachycardia, hypertension and impotence; and spontaneous and evoked myoclonus, among other neurological symptoms.

FFI presents a high heterogeneity and regional dependence regarding PrP depositions, gliosis and neuronal loss. In addition to disease heterogeneity a limited access to FFI cases, due to its low prevalence, hampers the knowledge about the biochemical and neuropathological alterations occurring in the disease.

In the present study, post-mortem samples of the entorhinal cortex, cerebellum and thalamus of well-characterized cases of FFI were analyzed. The aim was to shed light into the pathological and molecular features regarding neuronal and synaptic affection, neuroinflammation and aberrant prion protein features, such as expression levels, seeding activity and presence of misfolded structures.

CROSS TALK ON THE ROLE OF PARK7 (DJ-1) IN PATHOPHYSIOLOGY OF SPORADIC CREUTZFELDT-JAKOB DISEASES (sCJD) IN CEREBELLUM IN MM1 AND VV2 SUBTYPES

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PARK7 (also known as DJ-1) is well known for its role in the development of familial Parkinson's disease (*f*PD). Point mutations in PARK7 resulting in the loss of its cytoplasmic function lead to early onset of PD as well. On the contrary, PARK7 under the effect of oxidative stress has a protective role. PARK7 is oxidized in order to quench the reactive oxygen species (ROS) to protect neurons from death due to oxidative stress. Oxidized PARK7 is already observed in brains of PD and AD patients. In this study, however we; observed a significant expression of PARK7 in MM1 and VV2 subtypes in cerebellum part of the brain of sporadic Creutzfeldt-Jakob disease (sCJD) patients. sCJD is an idiopathic human prion disease with spongiform degeneration in the central nervous system. MM1 and VV2 subtypes are the two most prevalent subtypes of sCJD. During the whole differential proteome characterization of cerebellum part of the brain of MM1 and VV2 subtypes of sCJD cases by using 2-Dimensional gel electrophoresis, we found the upregulation of PARK7 in MM1 and VV2 subtypes of sCJD cases as identified by MALDI-TOF MS/MS and validated at protein level by western blot and mRNA level by qPCR. Up-regulation of PARK7 accounts for its role in protection against oxidative stress during the course of the disease in MM1 and VV2 subtypes of sCJD but this still needs to be elucidated. Oxidative stress is found to be the third top most regulated cellular mechanism during the pathophysiology of sCJD in our data. So oxidative stress can be considered as one of the major triggering factor along with others in the development of sCJD and other neurodegenerative diseases and PARK7 can be a potential diagnostic marker during the development of oxidative stress in various neurodegenerative diseases including sCJD.

SHORT ORAL COMMUNICATIONS

DEREGULATION OF MITOCHONDRIAL METABOLISM, PROTEIN SYNTHESIS AND PURINE METABOLISM IN CREUTZFELDT-JAKOB DISEASE

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It is widely accepted that cellular energy metabolism failure and mitochondrial damage, as well as alterations in protein synthesis occur in the course of several neurodegenerative diseases. In addition, through transcriptomic and metabolomic studies, we have recently observed a marked deregulation of purine metabolism in Alzheimer disease. The aim of our current work is to evaluate the vulnerability of mitochondrial function, and more specifically, of the electron transport chain (ETC), the protein synthesis machinery and purine pathways to sporadic Creutzfeldt-Jakob disease (sCJD).

For this purpose, we have analyzed the mRNA levels of 11 genes encoding subunits belonging to the five protein complexes of the ETC and 9 genes coding for proteins involved in energy metabolism, 21 genes coding for nucleolar, rRNA and ribosomal proteins participating in protein synthesis, and 23 genes encoding enzymes of the purine metabolism pathway, in the frontal cortex area 8 of 15 cases of sCJD subtype MM1 compared to age-matched controls. We have validated these results by assessing protein levels of distinct subunits from the ETC and measuring the protein levels of initiation and elongation factors.

Our studies revealed significant transcript down-regulation of genes encoding subunits of the five ETC complexes and other genes involved in energy metabolism in sCJD in comparison with control samples. Protein products of these genes are consistently diminished in sCJD MM1. We also found modifications in mRNA levels of genes related to protein synthesis and purine metabolism. Our data suggest an impairment of the ETC system and of protein synthesis, as well as of purine metabolism in CJD MM1.

TAUPATÍA CON RASGOS DE DEGENERACIÓN CORTICOBASAL EN LA ENFERMEDAD DE CREUTZFELDT-JAKOB: PERFIL NEUROPATOLÓGICO Y CLÍNICO EN UNA SERIE DE CASOS DE BANCO DE CEREBROS

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Introducción

Se ha relacionado que ciertas enfermedades neurodegenerativas asociadas a depósito de tau y otras proteínas comparten similitudes con los priones en cuanto a mecanismos moleculares y su posible transmisibilidad. En las enfermedades priónicas el depósito de tau se ha descrito en formas genéticas poco frecuentes y en la enfermedad de Gerstmann-Sträussler-Scheinker, pero no se considera parte del cuadro neuropatológico de la enfermedad de Creutzfeldt-Jakob (ECJ).

Materiales y métodos

Para investigar la coexistencia de ambas patologías y ver como modifica el perfil neuropatológico y clínico de la ECJ, se ha realizado inmunohistoquímica con anti -tau (AT-100), anti-tau (4R) y anti-AB amiloide en áreas isocorticales, estriado y tronco de 150 casos con diagnóstico neuropatológico de ECJ.

Resultados

Ocho casos con rasgos de Degeneración Corticobasal (DCB), uno acompañado de patología tipo Alzheimer y otro de granos argirófiros. Cinco corresponden a varones y 3 a mujeres. Polimorfismo del codón 129: MM (3), MV (2), VV (1). Edad media de presentación: 70 años. Tiempo de evolución: 5 meses. El cuadro clínico comenzó con problemas psiquiátricos o alteraciones de la marcha en 7 casos. Prot. 14.3.3: positiva en 7. El cuadro histológico y los depósitos de PrP res son los habitualmente observados en cada subtipo genético. La patología tau es con rasgos de DCB con astrocitos positivos, "pseudoplacas", neuronas positivas y escasos *"coiled bodies"*, en neocorteza y estriado.

Conclusiones

Patología tau con rasgos de DCB: 5 % en la serie estudiada.
Perfil incompleto de DCB y baja frecuencia de otra patología tipo tau asociada. Esto puede deberse a la inhibición de algún factor implicado en la agregación de tau.
Se encuentra tanto en ECJe como ECJf, sin modificar el fenotipo neuropatológico ni los depósitos de PrPres.
Menor tiempo de evolución. Más frecuente en varones.
Síntomas clínicos de inicio con trastornos psiquiátricos o alteraciones de la marcha fueron los más frecuentes.

MORPHOLOGICAL APPROACH TO GLIAL INVOLVEMENT IN PRION AND A PRION-LIKE DISEASES

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Currently, it has been impossible to develop preventive or/and curative treatments against prion and prion-like diseases. Gliosis is one of the histopathological insults found in the whole affected patients join to neuronal degeneration and deposits of aberrant protein. To our knowledge, there are not enough works focused on the host protection at cellular level in order to be used as an approach to pathogenesis of the neurodegeneration and as consequence, to establish a potential therapeutic target.

In this preliminary study, intensity and morphology changes in astroglial and microglial cells as a part of the host protective system are comparatively assessed in the cerebellum from Creutzfeldt-Jakob and Alzheimer's disease patients.

Glial cells have demonstrated to have a real relevance in the neurological deterioration. This morphological approach intends to deal with the involvement of glia, in interaction with neurons, in the neurodegenerative progress.

NOVEL ASSAY FOR DETECTION OF CSF 14-3-3γ LEVELS INCREASES sCJD DIAGNOSIS ACCURACY

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Introduction: Protein 14-3-3 is a reliable biomarker of rapid neuronal damage, specifically increased in sporadic Creutzfeldt-Jakob disease (sCJD) patients. Its detection is usually performed by Western Blot (WB), prone to methodological issues and leading to inconclusive results. In order to improve biochemical diagnosis of sCJD, efforts have been made to develop a quantitative assay.

Aim: This study aims to evaluate diagnostic performance of a recently developed quantitative enzyme linked immunosorbent (ELISA) assay for 14-3-3γ and to compare it with WB technique.

Methods: CSF samples from 145 patients with suspicion of prion disease, further classified as definite sCJD (n=72) or non-prion disease (Non-CJD; n=73) were included in this study. 14-3-3 protein was determined by both WB and 14-3-3γ ELISA assay (CircuLex™). Other neurodegeneration biomarkers (Tau and p-Tau) were evaluated by ELISA (Innogenetics®) and prion protein gene *(PRNP* codon 129) polymorphism assessed.

Results: 14-3-3γ levels were significantly increased (p<0,001) in sCJD (141740 ± 109157 LU) compared to non-CJD patients (6079 ± 7950 LU), showing very good accuracy to differentiate groups (AUC = 0.982; sensitivity =97%; specificity =94%). ELISA matched WB results in 82% of all cases, reaching 88% in *PRNP 129* homozygous patients. It also strongly correlated with Tau and pTau levels (p<0,0001), showing higher specificity than any other test (14-3-3 WB – 68%; Tau - 90%; pTau/Tau ratio - 88%). Surprisingly, ELISA levels were not significantly different between genotypes. From WB borderliners (n=44), ELISA correctly classified 41 patients (93%). Additionally, logistic regression analysis pointed 14-3-3 ELISA as the best predictive marker for sCJD (overall accuracy= 95%).

Conclusion: Despite specificity for 14-3-3γ isoform, results from ELISA not only match WB evaluation but also help further discriminating inconclusive results. Moreover, its levels seem independent from *PRNP* genotype, reinforcing this quantitative assay as a first screening test, allowing higher sample throughput and unequivocal results.

NETWORKING

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