



## Prion strain evolution and adaptation

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## **Book of Abstracts**

# Oral communications:

## Opening plenary lecture:

### O1 A unified view of prion biology and diseases

Stanely Prusiner

Institute for Neurodegenerative Diseases, University of California, San Francisco, USA.

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## Invited lectures:

### O2 Unusual types of CWD in Norwegian cervids

Sylvie L. Benestad (1), Tran L (1), Saure B (1), Terland R (1), Svendsen S (1), Dessen K (1), Haugum M (1), Handeland K (1), Madslie K (1), Kolbjørnsen Ø (1), Wisløff H (1), Moldal T (1), Våge J (1), Pirisinu L (2), Vaccari G (2), Bian J (3), Moreno JA (3), Kim S (3), Spraker T (4), Andreoletti O (5), Telling GC (3), Nonno R (2), Vikøren T (1)

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Chronic Wasting Disease (CWD) is a Transmissible Spongiform Encephalopathy (TSE) affecting cervids. TSEs, also called prion diseases, are invariably fatal and transmissible neurodegenerative diseases of humans and animals, also including scrapie in sheep and goat, bovine spongiform encephalopathy (BSE) in cattle, and Creutzfeldt-Jakob disease in humans. TSEs are characterized by the misfolding of the normal host-encoded cellular prion protein (PrP<sup>C</sup>) into an abnormal disease-associated isoform (PrP<sup>Sc</sup>). Classical scrapie of small ruminants and CWD of cervids have many common features, among them, to be contagious, spreading directly between animals or indirectly via environmental contamination. Since the first description in captive deer in Colorado in 1967, CWD is spreading to new states in North America. CWD is also detected in South Korea after importation of infected elk/wapiti (*Cervus canadensis*) from North America. More recently, a moose has been detected with prion disease in Finland.

In 2016, CWD was reported for the first time in Europe in wild reindeer (*Rangifer tarandus*), a species never previously found to be naturally infected. The biochemical analysis and the immunohistochemical (IHC) distribution of PrP<sup>Sc</sup> from Norwegian reindeer did not reveal differences with North American CWD isolates. However preliminary transmission results

suggest that the reindeer strain characteristics are different from those causing CWD in North-America.

In an attempt to limit the spread of the disease, the reindeer population in the affected area of Norway (Nordfjella) was culled and analysed for PrPSc. Totally, 19 reindeer were found positive out of 2471 tested in the Nordfjella population. A comprehensive nationwide surveillance program for cervids is ongoing and until now no additional reindeer cases have been detected outside the Nordfjella area.

Beside the detection of CWD in reindeer, four additional cases of prion disease have been detected in Norway, three in moose (*Alces alces*) and one in red deer (*Cervus elaphus*), all four in older animals. From current biochemical and IHC analyses, the moose cases were clearly distinguishable both from the Norwegian reindeer and from North American CWD cases. The preliminary transmission results suggest that the moose can be affected by a novel, atypical type of CWD, designated Nor16CWD.

The positive red deer showed characteristics that were close to the moose prion type, but not identical, and further studies are ongoing to characterise the prion type more specifically.

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### **O3 Experimental studies on prion transmission barrier and TSE pathogenesis in large animals**

Rosa Bolea(1), Acín C(1)Marín B(1), Hedman C(1), Raksa H(1), Barrio T(1), Otero A(1), LópezPérez O(1), Monleón E(1),Martín-Burriel(1), Monzón M(1), Garza MC(1), Filali H(1),Pitarch JL(1), Garcés M(1), Betancor M(1), GuijarroIM(1), GarcíaM(1), Moreno B(1),Vargas A(1), Vidal E(2), Pumarola M(2), Castilla J(3), Andréoletti O(4), Espinosa JC(5), Torres JM(5), Badiola JJ(1).

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Experimental transmission of Transmissible Spongiform Encephalopathies (TSE) has been understood and related with several factors that could modify the natural development of these diseases. In fact, the behaviour ofthe natural disease does not match exactly in each animal, being modified by parameters such as the age at infection, the genotype, the breed or the causative strain. Moreover, different TSE strains can target different animal species or tissues, what complicate the prediction of its transmissibility when is tested in a different species of the origin source. The aim of theexperimental studies in large animals is to homogenize all those factors, trying to minimize as much as possible variations between individuals. These effects can be flattened by experimental transmission in mice, in which a specific strain can be selected after several passages. With this objective, several experimental studies in large animals have been developed by the presenter research team.

Classical scrapie agent has been inoculated in cow, with the aim of demonstrate the resistance or susceptibility of this species to the first well known TSE;Atypical scrapie has been inoculated in sheep (using several routes of infection), cow and pig, with the objective of evaluating the potential pathogenicity of this strain; Classical Bovine Spongiform Encephalopathy (BSE) has been inoculated in goats aiming to demonstrate if the genetic background of this species could protect against this strain; goat BSE and sheep BSE have been inoculated in goats and pigs

respectively to evaluate the effect of species barrier; and finally atypical BSE has been inoculated in cattle to assess the transmissibility properties of this newly introduced strain.

Once the experiments have been carried out on large animal species, a collection of samples from animals studied were inoculated in different types of tg mice overexpressing PrP<sup>Sc</sup> in order to study the infectivity of the tissues, and also were studied using PMCA.

In summary, the parameters that have been controlled are the species, the strain, the route of inoculation, the time at infection, the genotype, the age, and the environmental conditions. To date, eleven of the atypical scrapie intracerebrally inoculated sheep have succumbed to atypical scrapie disease; six pigs to sheep BSE; one cow to classical scrapie; nine goats to goat BSE and five goats to classical BSE. PrP<sup>Sc</sup> has been demonstrated in all cases by immunohistochemistry and western blot.

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#### **O4 Prion forming domains in cell death signaling**

Sven Saupe

Institute of Chemistry and Biology of Membranes and Nanoobjects, Institut Européen de Chimie et Biologie, CNRS UMR 5248, Université de Bordeaux, Pessac, France.

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## **Selected oral presentations:**

#### **O5 Prion Disease in Dromedary Camels**

Babelhadj B (1), Di Bari MA (2), Pirisinu L (2), Chiappini B (2), Gaouar SB (3), Riccardi G (2), Marcon S (2), Agrimi U (2), Nonno R (2), Vaccari G (2)

(1) École Normale Supérieure Ouargla. Laboratoire de protection des écosystèmes en zones arides et semi arides University Kasdi Merbah Ouargla, Ouargla, Algeria; (2) Istituto Superiore di Sanità, Department of Food Safety, Nutrition and Veterinary Public Health, Rome, Italy  
(3) University Abou Bekr Belkaid, Tlemcen, Algeria.

Prions are responsible for fatal and transmissible neurodegenerative diseases including Creutzfeldt-Jakob disease in humans, scrapie in small ruminants and bovine spongiform encephalopathy (BSE). Following the BSE epidemic and the demonstration of its zoonotic potential, general concerns have been raised on animal prions.

Here we report the identification of a prion disease in dromedary camels (*Camelus dromedarius*) in Algeria and designate it as Camel Prion Disease (CPD). In the last years, neurological symptoms have been observed in adult male and female dromedaries presented for slaughter at the Ouargla abattoir. The symptoms include weight loss, behavioral abnormalities and neurological symptoms such as tremors, aggressiveness, hyper-reactivity, typical down and upwards movements of the head, hesitant and uncertain gait, ataxia of the hind limbs, occasional falls and difficult getting up. During 2015 and 2016, symptoms suggestive of prion disease were observed in 3.1% of 2259 dromedaries presented at ante-mortem examination. Laboratory

diagnosis was obtained in three symptomatic dromedaries, sampled in 2016 and 2017, by the detection of typical neurodegeneration and disease-specific prion protein (PrP<sup>Sc</sup>) in brain tissues.

Histopathological examination revealed spongiform change, gliosis and neuronal loss preferentially in grey matter of subcortical brain areas. Abundant PrP<sup>Sc</sup> deposition was detected in the same brain areas by immunohistochemistry and PET-blot. Western blot analysis confirmed the presence of PK-resistant PrP<sup>Sc</sup>, whose N-terminal cleaved PK-resistant core was characterized by a mono-glycosylated dominant form and by a distinctive N-terminal cleavage, different from that observed in BSE and scrapie.

PrP<sup>Sc</sup> was also detected, by immunohistochemistry, in all sampled lymph nodes (cervical, prescapular and lumbar aortic) of the only animal from which they were collected.

The PRNP sequence of the two animals for which frozen material was available, showed 100% nucleotide identity with the PRNP sequence already reported for dromedary camel.

Overall, these data demonstrate the presence of a prion disease in dromedary camels whose nature, origin and spread need further investigations. However, our preliminary observations on the rather high prevalence of symptomatic dromedaries and the involvement of lymphoid tissues, are consistent with CPD being an infectious disease. In conclusion, the emergence of a new prion disease in a livestock species of crucial importance for millions of people around the world, makes urgent to assess the risk for humans and to develop policies able to control the spread of the disease in animals and to minimize human exposure.

**See also WA10**

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## **O6 Different Chronic Wasting Disease strains circulate in North America and Europe**

Nonno R (1), Di Bari M (1), Pirisinu L (1), D'Agostino C (1), Vanni I (1), Vaccari G (1), Marcon S (1), Riccardi G (1), Viøren T (2), Våge J (2), Madslien K (2), Mitchell G (3), Benestad SL (2) and Agrimi U (1)

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Chronic Wasting Disease (CWD) was detected for the first time in Europe in wild Norwegian reindeer (*Rangifer tarandus*) and moose (*Alces alces*) in 2016. The disease in reindeer revealed a pattern similar to known CWD cases from North America; in contrast, CWD in moose presented with several unusual features, including old age (13-to-14 years) and absence of PrP<sup>Sc</sup> deposition in lymphoid tissues. Moreover, the biochemical and immunohistochemical features of PrP<sup>Sc</sup> in Norwegian moose were different from the Norwegian reindeer and unprecedented for CWD, suggesting that it could be a new CWD strain. In an earlier study we showed that North American CWD from elk, white-tailed deer and mule deer transmitted to bank voles carrying isoleucine at PrP codon 109 (Bv109I). This resulted in all CWD sources producing the same easily recognized vole-adapted CWD strain, typified by unprecedented short survival times of ~35-40 days, discrete distribution of spongiosis and relatively low levels of PrP<sup>Sc</sup>. The strain properties of CWD isolates from Norwegian moose (n=2) and reindeer (n=1), as well as from a Canadian moose, were therefore investigated by bioassay in Bv109I and compared with the previously characterized North American isolates.

The Canadian moose successfully transmitted on first (~220 dpi) and second passage (~40 dpi), reproducing the same vole-adapted strain previously isolated from other North American CWD sources. In sharp contrast, moose from Norway transmitted with longer survival time on first passage (~300-430 dpi), and induced pathological patterns and PrP<sup>Sc</sup> types different from those observed in Bv109I inoculated with North American CWD isolates. Second passage, available from one Norwegian moose, resulted in a new vole-adapted CWD strain, characterized by a survival time of ~80 dpi and by brain distribution of spongiosis and PrP<sup>Sc</sup> different from any previously characterized CWD isolate. Accordingly, the PrP<sup>Sc</sup> type reproduced in Bv109I was different from that observed after inoculation of Canadian moose and reminiscent of the PrP<sup>Sc</sup> type in the original Norwegian moose samples. Finally, the reindeer isolate failed to transmit to Bv109I so far (>600 dpi).

Overall, these data demonstrate that Norwegian moose are affected by a putatively new CWD strain, different from the CWD epidemic strain circulating in North American cervids. Lack of transmission, so far, of the reindeer isolate might depend on a low infectivity titer or could indicate that still another CWD strain affects reindeer. Further experiments are underway to address this issue. In conclusion, transmission and characterization in Bv109I of CWD isolates from Norway and North America failed, so far, to identify common CWD strains. This may have critical implications for understanding the origin of CWD epidemic in Europe and for designing of the most appropriate control strategies.

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## **O7 The strain properties of prions causing chronic wasting disease in Norwegian cervids are distinct from those causing disease in North America**

Bian J (1), Moreno JA (1), Kim S (1), Vikøren T (2), Madslie K (2), Våge J (2), Benestad SL (2), Telling GC (1)

(1) Colorado State University, Prion Research Center and the Department of Microbiology, Immunology & Pathology, Fort Collins, USA (2) Norwegian Veterinary Institute, Oslo, Norway.

Chronic wasting disease (CWD) is a burgeoning global epidemic of deer, elk and other cervids. The only known prion disease of wild animals, its contagious spread in North America appears irrevocable, with 24 States and two Canadian provinces reporting disease in farmed and/or free-ranging mule deer (*Odocoileus hemionus*); black-tailed deer (*Odocoileus columbianus*); white-tailed deer (*Odocoileus virginianus*); Rocky Mountain elk (*Cervus elaphus nelsoni*); and Shiras's moose (*Alces alces shirasi*). Imported North American CWD spread from farmed to novel native species in South Korea, specifically red deer (*Cervus elaphus*); sika deer (*Cervus nippon*); and offspring of crosses between red and sika deer. In 2016, CWD was identified in a wild Norwegian reindeer (*Rangifer tarandus tarandus*). Of the ~ 40,000 currently tested Norwegian cervids, 18 reindeer, three moose (*Alces alces alces*) and one red deer (*Cervus elaphus*) have been diagnosed with CWD. In March 2018, Finland also recorded a case of CWD in a wild moose.

We previously developed prototype, CWD-susceptible Tg mice. Tg(Q226) express cervid PrP with glutamine (Q) at residue 226, the genotype of deer, reindeer, and moose, while Tg(E226) express glutamate (E) at this position, which is the genotype found in elk. More recently, we developed gene-targeted (Gt) mice in which expression of cervid PrP with E or Q at 226 faithfully recapitulates that of native host PrP<sup>C</sup> in an FVB/N background. Our analyses in Tg mice of the transmission properties of CWD prions causing disease in North American deer and elk allowed us to define two predominant strain types, referred to as CWD1 and CWD2, with distinct incubation times and resultant neuropathological profiles. Here we report our preliminary findings of the transmission properties of Norwegian CWD prions in Tg and Gt mice. These analyses show

that the properties of prions causing CWD in Norwegian moose are different from those causing CWD in Norwegian reindeer, and that the strain properties of Norwegian CWD prions bear no relationship to those of CWD prions causing disease in North American moose, deer and elk. Our findings increase uncertainty surrounding the ecological and economic consequences of CWD, and raise the possibility for these emergent strains to cause novel prion diseases in further species, including humans.

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## **O8      Gene targeted mice reveal an essential role for PrP residue 226 and an accurate means to model CWD peripheral pathogenesis**

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In previous studies, we developed prototype Tg mice that recapitulated major aspects of chronic wasting disease (CWD) pathogenesis. These include Tg(Q226), which express cervid PrP with glutamine (Q) at residue 226, the genotype of deer, reindeer, and moose, and Tg(E226) which express glutamate (E) at this position, the genotype found in elk. In addition to overcoming restrictive drawbacks of studying CWD transmission in natural hosts, the development of CWD-susceptible Tg mice represented a significant step forward for understanding CWD pathogenesis, the mechanism of contagious transmission, and the prevalence of CWD strains. Here we report on the development and characterization of gene-targeted (Gt) mice expressing cervid PrP with either E or Q at residue 226, [Gt(E226) and Gt(Q226)]. Gt(E226) express the wild type PrP primary structure from elk, while Gt(Q226) express wild type PrP from deer, moose and reindeer. Expression in Gt mice faithfully recapitulates that of native host PrP<sup>C</sup> in an FVB/N background. We report that CWD incubation times are consistently faster in Gt(E226) and Gt(Q226) mice. Residue 226 not only controls the tempo but also the neuropathological consequences of disease. The availability of Gt mice on an inbred FVB background not only enabled us to model heterozygosity at residue 226, but also to precisely determine the effects of gene dose, and mouse PrP expression on pathogenesis. In Gt mice, PrP expression and tissue distribution are accurately controlled, and peripheral challenge is surprisingly effective. Brain distribution of PrP<sup>Sc</sup> in orally dosed and ip inoculated Gt(E226) were similar, and distinctly different from ic inoculated Gt(E226). Cohousing of uninfected with CWD-challenged mice also demonstrated that Gt mice recapitulate the remarkably efficient horizontal transmission of CWD observed in natural hosts.

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## **O9      Permeability of the bovine transmission barrier to Atypical/Nor98 scrapie**

Huor A. (1), Vidal E.(2), Espinosa JC (3), Lacroux C.(1), Cassard H.(1), Douet JY.(1), Lugan S.(1), Aron N.(1), Tillier C.(1), Bolea R.(4), Benestad SL.(5), Orge L.(6), Torres JM.(3), and Andréoletti O(1)

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Atypical/Nor98 Scrapie has been identified in many countries, including Australia and New Zealand. In the EU small ruminants' population, its prevalence was estimated to range between 5 to 8 positive small ruminants per 10,000 tested per year.

The zoonotic potential and the risk that atypical scrapie might represent for other farmed animal species remains unknown.

In this study we investigated the capacity of a panel of Atypical scrapie isolates (n= 8 issued four different countries) to propagate in bovine PrP expressing mice (tgBov).

The inoculation in tgBov of all the selected isolates resulted in Prion propagation. Surprisingly the properties of the TSE agents recovered in tgBov were dramatically different from those present in the original isolates. Their in-depth phenotypic characterization (bioassay in various reporter models, PrP<sup>res</sup> biochemistry) indicated that atypical scrapie passage through the cattle transmission barrier resulted, in the majority of the cases, in the emergence of classical BSE. Investigations carried-out using highly sensitive in vitro amplification of Prion (PMCA) confirmed the absence of any detectable classical BSE prions in the original isolates.

Our findings suggest that cattle exposure to atypical scrapie could be responsible of the occurrence of classical BSE in this species. These results also raise some concerns about the current and future changes in the protection measures that were implemented to mitigate animal and human exposure to TSE agents.

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## **O10 Zoonotic potential of atypical BSE prions: a systematic evaluation**

Marín-Moreno A (1), Espinosa JC (1), Douet JY (2), Aguilar-Calvo P (1), Piquer J (1), Lorenzo P (1), Lacroux C (2), Huor A (2), Lugan S (2), Tillier C (2), Andreoletti O (2) and Juan María Torres (1)

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Bovine Spongiform Encephalopathy (BSE) is the only zoonotic prion recognized to date. The transmission of BSE to humans caused the emergence of variant Creutzfeldt-Jakob disease (vCJD). In 2004 two new atypical prion agents were identified in cattle: H- and L- BSE prion strains.

The zoonotic potential of atypical BSE prions was assessed by inoculating three different isolates of cattle H- and L-BSE in transgenic mouse lines that overexpress the human PrP covering the three different genotypes of the aminoacid 129 (TgMet<sub>129</sub>, TgMet/Val<sub>129</sub> and TgVal<sub>129</sub>). This polymorphism is known to be a key element involved in human resistance/susceptibility to BSE. In addition, TgMet<sub>129</sub> and TgVal<sub>129</sub> were challenged with one H- and L-BSE isolates adapted to sheep PrP expressing hosts to assess if intermediate passage in sheep could modify the capacity of these prions to cross the human species barrier.

Our results confirm that L-BSE transmits to TgMet<sub>129</sub> even better than epidemic BSE. However, atypical L-BSE agent was unable to infect TgVal<sub>129</sub> or TgMet/Val<sub>129</sub> mice, even after passage in TgMet<sub>129</sub>. No transmission was observed with H-BSE in any mice model inoculated, irrespectively of the 129 polymorphism. After passage in sheep PrP expressing host, the properties of both H and L-BSE including their capacity to cross the human species barrier were dramatically

affected, emerging prion strains features that resemble those of sporadic Creutzfeldt-Jakob disease (sCJD).

To date, this is the more extensive and complete analysis of the zoonotic potential of atypical BSE prions. These results advise not to ignore the zoonotic potential of these agents.

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## **O11 Preclinical detection of prions in skin samples of scrapie-infected animals by serial PMCA and RT-QuIC assays**

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A definitive pre-mortem diagnosis of prion diseases is dependent on available brain biopsy tissues for prion detection at present and no validated preclinical prion diagnosis has been reported to date. Our recent study has revealed that infectious prions are detectable in the skin of cadavers with sCJD and vCJD. To determine the feasibility of using skin for preclinical diagnosis, we examined skin samples from different body areas of animal models including the 263K-infected hamsters and the sCJDDMM1-infected humanized Tg40h mice at different time-points after intracerebral inoculation utilizing two highly sensitive serial protein misfolding cyclic amplification (sPMCA) and real-time quaking-induced conversion (RT-QuIC) assays. Clinical signs were observed at week 11 in hamsters and month 6 in Tg40h mice post inoculation. PrP<sup>Sc</sup> and spongiform degeneration became detectable in the brain at 4 and 7 weeks for hamsters and at 5 months for Tg40h using conventional Western blotting and histology. In contrast, skin PrP<sup>Sc</sup> was detected by sPMCA as early as 2 weeks for hamsters and 1 month for Tg40h post inoculation; prion-seeding activity was also detected by RT-QuIC assay in infected hamster skin at 3 weeks and Tg40h mouse skin at 5 months. While sPMCA detected hamster skin PrP<sup>Sc</sup> at least one-week earlier than did RT-QuIC, a time-dependent increase in the skin PrP<sup>Sc</sup> from the early stage to the terminal stage of prion infection was more obvious with RT-QuIC than with sPMCA. Moreover, sPMCA amplified PrP<sup>Sc</sup> in the skin of PBS-inoculated negative controls co-habitated with the 263K-inoculated hamsters at 2 weeks post-inoculation while RT-QuIC exhibited positive prion-seeding activity in the skin of negative controls co-habitated with infected hamsters only at the terminal stage. These data suggest that skin prions could be used as a biomarker for preclinical diagnosis of prion diseases and monitoring disease progress and combination of both sPMCA and RT-QuIC assays may be helpful in differentiating the origins of skin prions.

See also poster P89

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**O12 Multiple system atrophy prions replicate in Tg(*SNCA*<sup>A53T</sup>) mice and induce glial pathology throughout the limbic system**

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In a series of recent publications, multiple system atrophy (MSA) was identified as a prion disease following the transmission of neurological symptoms to transgenic mice expressing A53T mutant human  $\alpha$ -synuclein (TgM83<sup>+/-</sup>). Here we inoculated mice expressing wild-type (WT), A30P, and A53T human  $\alpha$ -synuclein on a mouse knockout background [Tg(*SNCA*), Tg(*SNCA*<sup>A30P</sup>), and Tg(*SNCA*<sup>A53T</sup>), respectively]. Unlike studies using TgM83<sup>+/-</sup> mice, motor deficits were not observed in animals expressing WT or mutant  $\alpha$ -synuclein 400 and 330 days post inoculation, respectively. However, brain homogenates from the Tg(*SNCA*<sup>A53T</sup>) mice contained  $\alpha$ -synuclein prions, as measured in the  $\alpha$ -syn140\*A53T-YFP cell assay, and showed a similar biological activity profile to MSA prions in a panel of  $\alpha$ -synuclein-YFP cell lines. Moreover, these mice developed hyperphosphorylated  $\alpha$ -synuclein neuropathology throughout the limbic system, particularly in the hippocampus and entorhinal cortex, which co-localized with antibodies for glial cells following immunostaining. Importantly, this pathology is distinct from the induced pathology in the TgM83<sup>+/-</sup> mice, which develop neuronal  $\alpha$ -synuclein aggregates throughout the hindbrain causing motor deficits with advanced disease. In contrast, the Tg(*SNCA*<sup>A53T</sup>) mice have no  $\alpha$ -synuclein accumulation in the cerebellum or brainstem. Brain homogenates from Tg(*SNCA*<sup>A53T</sup>) mice inoculated with two MSA cases or one control sample were then used to inoculate TgM83<sup>+/-</sup> animals. Serial passaging of MSA induced motor dysfunction and  $\alpha$ -synuclein prion formation in the TgM83<sup>+/-</sup> mice, but the control sample had no effect on the animals. The confirmed transmission of  $\alpha$ -synuclein prions to a second synucleinopathy model and the ability to propagate the prions between two distinct mouse lines with retention of strain properties provides definitive evidence that MSA is a prion disease.

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**O13 Amyloid polymorphisms constitute distinct clouds of conformational variants in different etiological subtypes of Alzheimer's disease**

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Alzheimer's disease (AD) pathogenesis is widely believed to be driven by the pathological aggregation of the amyloid- $\beta$  peptide ( $A\beta$ ). Despite a common origin in the accumulation of the  $A\beta$  protein, the clinical and pathological phenotype of AD exhibits conspicuous variability among patients.

Recent work in humans suggests that  $A\beta$  can aggregate into structural variants with distinct pathobiological traits. By using extracted fibrils from AD brains to seed the aggregation of synthetic  $A\beta$ , it has been shown that  $A\beta$  assumes a single, dominant conformation within a given brain with structural heterogeneity among AD patients (Lu JX, et al., *Cell*, 2013, Qiang W, et al., *Nature*, 2017).

We used luminescent conjugated oligothiophenes (LCOs) to interrogate the molecular structure of  $A\beta$  in amyloid plaques from patients with etiologically distinct subtypes of AD. When bound to amyloid, LCOs yield fluorescence emission spectra that reflect the 3D structure of the protein aggregates (Aslund A, et al., *ACS Chem Biol*, 2009; Herrmann US, et al., *Sci Transl Med*, 2015).

LCO-stained cores of  $\beta$ -amyloid plaques in post-mortem tissue sections from frontal, temporal, and occipital neocortices in 40 cases of familial AD or sporadic AD were analysed. The spectral attributes of LCO-bound plaques varied markedly in the brain, but the mean spectral properties of the amyloid cores were generally similar in all three cortical regions of individual patients. Remarkably, the LCO amyloid spectra differed significantly among some of the familial and sporadic AD subtypes, and between typical patients with sporadic AD and those with posterior cortical atrophy AD. On the contrary, the spectral attributes of LCO-bound neurofibrillary tangles did not significantly differ among and within patients. Neither the amount of  $A\beta$  nor its protease resistance correlated with LCO spectral properties.

To support the observed conformational differences among the amyloid cores, experimental transmission studies were performed indicating that LCO spectral amyloid phenotypes could be partially conveyed to the induced  $A\beta$  plaques.

Our findings indicate that polymorphic  $A\beta$ -amyloid deposits within the brain cluster as clouds of conformational variants in different AD cases, whereas neurofibrillary tangles do not show polymorphism. Heterogeneity in the molecular architecture of pathogenic  $A\beta$  among individuals and in etiologically distinct subtypes of AD justifies further studies to assess putative links between  $A\beta$  conformation and clinical phenotype.

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#### **O14 Prion strain-like properties of misfolded superoxide dismutase-1 (SOD1)**

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Mutations in the Cu-Zn superoxide dismutase 1 (SOD1) gene are known to be causative in 10-20% of familial amyotrophic lateral sclerosis (fALS) cases. The duration of disease in these patients can vary, such that some mutations are associated with disease of less than 3 years, whereas others are associated with disease of more than 20 years. One potential explanation for this variability in disease progression is that distinct SOD1 mutations exhibit prion strain-like properties that may affect their ability to propagate within the CNS. To address this, we tested

various preparations containing different SOD1 mutations in in vivo and ex vivo seeding assays to investigate several SOD1 properties. We injected multiple misfolded SOD1-containing preparations within the spinal cords of mice at postnatal day P0. These recipient mice express low levels of the G85R variant of human SOD1 fused to YFP (G85R-SOD1:YFP) and are permissive for motor neuron disease induction. Once MND was induced, the spinal cords were analyzed for the presence and morphology of the induced G85R-SOD1:YFP aggregates by direct fluorescence. Additionally, as an ex vivo assay, these SOD1-containing preparations were added to organotypic spinal cord slice cultures prepared from G85R-SOD1:YFP mice to directly visualize the induced aggregates over time. We observed both distinct SOD1:YFP aggregate pathologies and incubation periods that bred true upon repeated passages. Additionally, we observed the same SOD1:YFP aggregate structures in spinal cord slice cultures as was seen in mice, when treated with a given SOD1 containing preparation. The distinct SOD1:YFP pathologies observed suggest prion strain-like properties for SOD1. Additionally, the fact that the morphology of these structures bred true upon second passage in mice with the same genotype suggests that the formation of these aggregates are produced by conformational templated misfolding, which is responsible for the propagation of prions. These results strongly suggest prion strain-like properties are involved in the misfolding and propagation of SOD1-linked ALS.

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## **O15 Redox regulation of adult neurogenesis by PrP and PrP N-terminal cleavage fragments**

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The ability to stimulate the production of new neurones (neurogenesis) is linked with adult memory formation, consolidation and loss. Adult neurogenesis requires both quiescent and actively proliferating pools of neural stem cells (NSCs). Actively proliferating NSCs ensure that neurogenic demand can be met, whilst the quiescent pool ensures NSC reserves do not become depleted. The processes controlling NSC quiescence and active growth cycles are only just beginning to be defined. Recently, the prion protein (PrP) was linked with enhanced NSC proliferative capacity. PrP is additionally linked with memory formation and consolidation, indicating PrP modulation of NSC growth has potentially functional outcomes. We investigated how PrP and two of its endoproteolytic cleavage fragments (N1 and N2) influence neurogenesis in vitro using murine NSCs harvested from the brains of adult mice. PrP knock-out NSCs showed increased cellular senescence (a resting state from which proliferation cannot be resumed) and decreased cellular energy, with concurrent increases in expression of the pro-oxidant enzyme NADPH oxidase-2 and the mitochondrial anti-oxidant enzyme superoxide dismutase-2 (SOD2). A lack of PrP expression also impaired differentiation into mature neurones in both 2D and 3D cultures. Over-expression of PrP stimulated increased proliferation and enhanced the number of neurones produced during differentiation. In contrast, the secretory N1 and N2 cleavage fragments demonstrated the opposite effect on NSC growth by decreasing proliferation, migration and neurite outgrowth. N1 and N2 switched NSCs from a proliferative to a quiescent (resting) state, without increasing senescence or decreasing viability. Quiescence was initiated by the PrP cleavage products through changing intracellular redox balance. Firstly, the N1 and N2 fragments reduce intracellular reactive oxygen species, with the N2 fragment appearing to directly reduce the activity of NADPH oxidase. The inhibition of redox signalling resulted in increased mitochondrial fission, which rapidly signalled quiescence. Thereafter, quiescence was maintained through downstream increases in the expression and activity of SOD2, which reduced mitochondrial superoxide. We further observed that PrP is predominantly cleaved in quiescent

cultured mouse NSCs and human brain NSCs indicating a dynamic role of PrP and its cleavage fragments in maintaining the growth cycle of adult NSCs.

**See also poster P147**

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## **O16 Autophagy has various roles in the life cycle of prions**

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Prion diseases are a family of fatal infectious neurodegenerative disorders characterized by the accumulation of the pathologic isoform of the normal cellular prion protein. Prion infection at the cellular level is the result of equilibrium between propagation, degradation and cellular release of prions, and involves various cellular machineries. Autophagy is a cellular program which can exert both beneficial and adverse effects in various neurodegenerative diseases. We tested the role of autophagy in prion infection and how this cellular machinery affects the life cycle of prions. One part of our work focused on induction of autophagic flux by chemical compounds to increase the cellular degradation of PrP<sup>Sc</sup>. This work provided robust scientific premise that drug-induced stimulation of autophagy has anti-prion effects *in vitro* and *in vivo*. Another part of our studies asked the question whether autophagy provides disaggregase function as needed for prion propagation. This was based on our findings in mouse embryonic fibroblasts which showed a pronounced dependence of prion replication on autophagy competence. However, in prion-infected neuronal cells autophagy had usually an opposite role. Cells ablated for autophagy competence by gene editing harbored elevated amounts of prions indicating neuronal cells use autophagy constitutively for prion degradation. This data shows that autophagy is used for at least two functions in the life cycle of prions, and depending on the cellular context this can be protective against or supportive of prion infection. The third aspect of our work addressed the role of autophagy in exosomal release of prions. Prions can be present in nano-vesicles called exosomes and it is likely that exosomes participate in delivering prions to uninfected neighboring cells and tissues. We addressed the link between autophagy, exosomes and release of prion infectivity using different cell culture models. Our results show a profound interplay between autophagy and exosome release, which influences lateral transmission of prions. Induction of autophagy decreases both the cellular level of prions and their exosomal release. This represents a dual target for therapeutic approaches to fight prion diseases. Overall, our studies describe novel impacts of autophagy on the life cycle of prions. This new understanding provides novel targets for therapy against prion diseases.

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## **O17 Evaluation of iatrogenic risk of CJD transmission associated with corneal transplantation**

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Sporadic Creutzfeldt–Jakob disease (sCJD) has been documented to be accidentally transmitted by contaminated corneal transplants. To date, only one case is considered as definite, while 5 other suspect cases are classified as probable or possible. However, the specific transmission risk associated with this widely-performed transplantation procedure has never been studied.

In this study, bioassays in transgenic mice expressing the human PrP confirmed the presence of infectivity in the cornea of 2 sCJD patients. Infectivity was also detected in other ocular tissues (optic nerve, retina, vitreous body, choroid and lacrimal gland) from one of these patients.

Based on these results, we investigated the presence of infectivity in the cornea of different TSE animal models. In conventional mice (RML strain) as well as in sheep (PG127 scrapie), infectivity could only be detected in the corneas collected at the late stage of the disease incubation phase.

In parallel to these experiments, corneas collected at different stages of the incubation period in infected mice and sheep were grafted into healthy recipients.

Our results showed that corneas collected during the late asymptomatic phase or in affected animals were able to transmit TSE infectivity. Importantly, after the death of the recipients (up to 2.5 years after surgery) infectivity could still be detected in the grafted cornea.

These data confirm the potential for sCJD transmission by corneal grafts. They also provide crucial data for the assessing the TSE transmission risk associated with various other ophthalmologic procedures.

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## **O18 Epigenetics in prion diseases: identification of novel markers for misfolded protein-associated neurodegenerative disorders**

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Prion diseases are fatal neurodegenerative diseases of humans and animals caused by the misfolding and aggregation of prion protein (PrP). Misfolded proteins play an important role in many forms of dementia but despite intensive efforts to better understand the pathophysiology of these diseases, the mechanisms involved are still poorly understood. Although neurodegeneration is affecting millions of people worldwide there is a huge unmet need for novel diagnostic and treatment approaches in neurodegenerative disorders to counter therapeutic nihilism in this area. Recent studies have implicated a role for genetic and epigenetic variation in neurodegenerative disorders. To date however no study has systematically explored the contribution of genetic and epigenetic changes in human prion diseases.

We identified 38 CpGs sites that showed significant differential DNA methylation in blood between cases and controls in the discovery set (227 samples), with Benjamini-Hochberg false discovery rate <0.05 after Bonferroni correction. In addition, we also identified one site whose levels of methylation correlates with disease stages and severity. Using RNA sequencing, we found 319 genes whose expression differentiate sCJD peripheral bloods from healthy controls; and 15 miRNAs significantly specifically deregulated in sCJD.

Integrating our data, we have established a sCJD specific signature, with genes between the two groups being enriched in functions related to neuroinflammation and immunity; and have correlated it with age of onset, disease progression, severity, decline, and genotype.

In addition, to identify molecular pathways associated with prion disease physiopathology, we present a framework for genome-wide studies of DNA methylation, integrating our data with results obtained from genome-wide association studies as well as other epigenetic marks profiles on the same samples.

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## **O19 Experimental transfusion of variant CJD-infected blood reveals novel class of prion disorders**

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The recently reevaluated high prevalence of healthy carriers (1/2000 in UK) of variant of Creutzfeldt-Jakob Disease (v-CJD), whose blood might be infectious, suggests that the evolution of this prion disease might not be under control as expected and enforces the necessity to assess the corresponding transfusional risk.

After experimental transfusion of macaques and conventional mice with blood derived from v-CJD exposed individuals, we confirmed in these both models the transfusional transmissibility of v-CJD in optimal conditions, corresponding to non-deleukocyted, whole blood samples derived from donors (of same species) with high levels of peripheral replication and sampled during the clinical phase. However, we also observed unexpected neurological syndromes transmissible by transfusion in a higher proportion of recipients, which occurred under less optimal conditions for prion transmission, which included deleukocyted samples, donors with low levels of peripheral replication, inter-specific (human to macaque or macaque to mice) transfusion and notably in two recipients transfused with blood derived from healthy carriers. Despite their prion etiology confirmed through transmission experiments, these original cases would escape classical prion diagnosis, notably in the quasi-absence of detectable abnormal PrP with current techniques. In conventional mice, incomplete vCJD phenotypes with spinal involvement were observed, whereas macaques developed an original, yet undescribed myelopathic syndrome associating demyelination and pseudo-necrotic lesions of spinal cord, brainstem and demyelination of the optical tract without affecting encephalon, which is rather evocative of spinal cord diseases than prion diseases.

These atypical profiles, that were also observed after intravenous exposure to soluble fractions of infected brains, were associated to an absence of detectable accumulation of abnormal PrP in follicles of lymphoid organs. Optimized techniques of immunohistochemistry identified an ectopic accumulation of abnormal PrP around splenic vessels in both primates developing vCJD or myelopathy. In parallel, those atypical syndromes were transmissible to immunocompromised mice through the intravenous route. Taken altogether, these results suggest that some prion variants, mostly associated to soluble prions, may follow distinct pathophysiological pathways that would be independent of the immune system. In parallel to the obvious consequences in terms of public health if such a situation should exist in humans, these observations questioned the possibility that the spectrum of prion diseases may extend the current field restricted to the phenotypes associated to protease-resistant PrP.

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## **O20 AP326, a new FDA-approved drug active against PrP<sup>Sc</sup>**



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Aim: Identify new anti-prion compounds and their mode of action.

Methods: We used an original screening process based on the SOSA (Selective Optimization of Side Activities of drug molecules (1)) approach to identify new anti-prion drugs. This screening strategy proposes to preferably screen drugs that are already in clinic or clinical trials in order to determine if they might have additional pharmacological targets. Because their toxicity, safety and bioavailability in humans have already been tested, the drugs issued from libraries based on the SOSA approach could rapidly be administered as compassionate therapeutics to people presenting symptoms of prion or prion-like diseases for which no treatment is currently available. FDA-approved drugs were first screened against [*PSI*<sup>+</sup>] *S. cerevisiae* yeast prion. The compounds able to cure [*PSI*<sup>+</sup>] were then tested in a secondary screen based on [URE3] (2). Given the great divergence of primary sequence and biochemical function of the two proteins at hand, molecules that are active against both prions are likely to act on conserved mechanisms controlling prion propagation. The activity of the positive hits has finally been evaluated against PrP<sup>Sc</sup> using *in vitro*, *ex vivo* and *in vivo* models.

Results: This screening process allowed us to identify a new antiprion drug called AP326\*. This FDA-approved drug is active against PrP<sup>Sc</sup> *in vitro* (MovS6 cell line) and *ex vivo* in an organotypic brain slice culture system. AP326 also increases the survival time of prion-infected Tg338 mice (3) in late treatment conditions, i.e., when treatment started 40 days post-infection. Finally, we found that AP326 inhibits the protein folding activity of the ribosome (PFAR) *in vitro*. PFAR, which is born by domain V of the large rRNA of the large subunit of the ribosome, corresponds to the second enzymatic activity of the ribosome, together with its peptidyl transferase activity (4). As we recently demonstrated that PFAR is involved in the propagation of [*PSI*<sup>+</sup>] in yeast (5), it suggests that PFAR is a potential therapeutic target for prion diseases.

Conclusions: AP326 is a FDA-approved drug, thus its therapeutic repositioning as an anti-prion compound towards clinic can be envisioned.

1-Wermuth C. G., *J Med Chem* 47:1303 (2004)

2- Bach et al., *Nat Biotechnol* 2003, 21(9):1075.

3- Archer F. *et al.*, *J Virol* 78:482 (2004)

4-Das D. *et al.*, *Biotechnol J* 3:999 (2008).

5- Blondel, M. *et al.* *Sci Rep* 6, 32117 (2016)

\* The identity of AP326 will be revealed by the time of PRION2018 congress.

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## **O21    Generation of novel neuroinvasive prions following intravenous challenge**

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Prion conformers differ in their capacity to penetrate the CNS from extraneural sites; certain fibrillar prions replicate persistently in lymphoid tissues with no CNS entry, leading to chronic silent carriers. There are an estimate 1:2000 subclinical carriers of variant Creutzfeldt-Jakob (vCJD) prions in the UK, and vCJD prions have been transmitted through blood transfusion, yet the types of circulating prion conformers that neuroinvade remain unclear. We investigated how prion conformation impacts the brain entry of transfused prions by challenging mice intravenously to subfibrillar and fibrillar strains. We show that most strains infiltrated the brain and caused terminal disease, however the fibrillar prions showed reduced CNS entry in a strain-dependent manner. Strikingly, highly fibrillar mCWD prions replicated in the spleen and emerged in the brain as a novel strain, indicating that a new neuroinvasive prion had been generated from a previous non-neuroinvasive strain. The new strain showed altered plaque morphology, brain regions targeted, and biochemical properties as compared to the original strain, and these properties were maintained upon intracerebral passage. Intracerebral passage of prion-infected spleen re-created the new strain and splenic prions resembled the new strain biochemically, collectively suggesting splenic prion replication as a potential source. Taken together, these results indicate that intravenous exposure to prion-contaminated blood or blood products or even other extraneural prion exposures may generate novel neuroinvasive prion conformers and disease phenotypes, potentially arising from prion replication in non-neural tissues or from conformer selection.

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## **O22    Epitope unmasking due to incomplete N-glycan attachment establishes that PrP<sup>Sc</sup> is underglycosylated relative to PrP<sup>C</sup> and affords a novel means of prion detection**

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Despite considerable efforts to develop disease-specific immunological reagents, purportedly PrP<sup>Sc</sup>-specific monoclonal (mAbs) ultimately failed to discriminate non-infectious from infectious PrP aggregates. Here we report an innovative approach for describing prion infectivity based on covalent structural differences between PrP isoforms, rather than dubious prion-specific conformational epitopes. We show that the epitope of mAb PRC7, which is comprised of tyrosine (Y) 154, glutamine (Q) 185, and phenylalanine (F) 197, is occluded when glycans attach to N196. Consequently, PRC7 recognizes underglycosylated PrP, comprised of 27-kDa unglycosylated and 30-kDa mono 1, referred to as PrP<sup>Gly-</sup>, but not mono 2 or diglycosylated PrP. Importantly, underglycosylated PrP is preferentially produced during prion infection, and the emergence of

PrP<sup>Gly-</sup> correlates with increasing prion titers. PrP<sup>Gly-</sup> shares properties with PrP<sup>Sc</sup>, namely, differential resistance to protease treatment, insolubility in non-denaturing detergents, cellular proteolytic processing, and kinetics of formation. PrP<sup>Gly-</sup> formation occurred in response to infection with various mouse-adapted scrapie prion strains, chronic wasting disease (CWD) of cervids, transmissible mink encephalopathy, and sheep scrapie. The overlapping properties of PrP<sup>Gly-</sup> and PrP<sup>Sc</sup> afforded a direct means to differentiate the conformational stability of prion strains without resorting to differential resistance to protease treatment. This highly sensitive approach enabled discrimination of prion conformers constituting CWD types 1 and 2 which had previously eluded definition. Our findings demonstrating post-translational differences between PrP<sup>C</sup> and PrP<sup>Sc</sup> not only offer an innovative framework to understand the mechanism of prion replication in which PrP under-glycosylation facilitates prion formation, but also satisfy a longstanding goal in the field of prion biology, namely the isolation of prion-specific immunological reagents.

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### **O23 Purified 7 kDa PrP<sup>res</sup> aggregates from GSS-A117V are infectious**

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We recently reported that Gerstmann-Sträussler-Scheinker disease (GSS) with the A117V mutation, characterized by a PrP<sup>Sc</sup> with a protease resistant core made of N and C termini cleaved PrP peptides of ~7 kDa (7 kDa PrP<sup>res</sup>), is transmissible in bank voles. However, definite proof of the infectious nature of this PrP<sup>Sc</sup> upon PK-treatment is still missing. We thus attempted to purify GSS-A117V 7 kDa PrP<sup>res</sup> in order to evaluate its role in terms of infectivity and strain features.

Brain homogenate from a human GSS-A117V case was either treated with PK or left untreated and then endpoint titrated in voles. The same case was subjected to a purification method that uses limited proteolysis with Pronase E (1) or PK, followed by centrifugations with an iodixanol cushion to obtain high levels of purified PrP<sup>Sc</sup>. The insoluble pellet from the Pronase E (P2-PronE) and the PK method (P2-PK), and the soluble supernatants (SN2-PronE and SN2-PK) were inoculated in voles.

GSS-A117V showed an extremely high infectious titer ( $10^{9.3}$ ) that was not affected by the PK treatment ( $10^{9.0}$ ), as it happens for classical prions. Given the high levels of PK-resistant infectivity, we used GSS-A117V as starting point for the purification of 7 kDa PrP<sup>res</sup>. Indeed, we were able to obtain a semi-purified P2-PronE fraction, which didn't contain the expected full length PrP<sup>Sc</sup> but 7 kDa PrP<sup>res</sup> accompanied by a small amount of higher MW fragments with intact N terminus. Upon PK treatment of this fraction (P2-PronE-PK), only 7 kDa PrP<sup>res</sup> was observed. As the yield of PrP<sup>res</sup> in P2 was much lower than that obtained with classical PrP<sup>Sc</sup>, we analyzed the intermediate fractions of the purification and compared the behavior of classical and atypical PrP<sup>Sc</sup>. The PrP<sup>Sc</sup> types behaved similarly during the first passages of the protocol, but diverged in the last centrifugation step; PrP<sup>res</sup> was distributed in SN2 and P2 at a ratio of ~1:2 and 3:1 for classical and atypical PrP<sup>Sc</sup> respectively. Comparable results, in terms of yield and SN2/P2 PrP<sup>res</sup> distribution, were obtained with the PK purification method. Only 7 kDa PrP<sup>res</sup> was recovered in P2-PK. Once inoculated in voles, the purified P2-PronE, P2-PronE-PK and P2-PK, as well as SN2-PronE and SN2-PK, were highly infectious, with infectivity levels that directly correlated with the 7 kDa PrP<sup>res</sup> amount detected in the fractions. All inoculated voles reproduced the same

prion strain as with the original unpurified GSS inoculum.

Our study demonstrates that purified aggregates of 7 kDa PrP<sup>res</sup> are fully infectious and encode the biochemical and biological strain features of the original sample. These findings imply that the C terminus of PrP<sup>Sc</sup> can be dispensable for infectivity and strain features. Therefore, this argues for 7 kDa PrP<sup>res</sup> aggregates, composed of nonglycosylated and non GPI-anchored PrP peptides spanning a.a. 90-150, as being reliably considered as fully competent “miniprions”, easily exploitable for structural studies.

(1) Wenborn et al., 2015.

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## **O24 Shaken, not sonicated: A strategy to generate large amounts of recombinant *bona fide* prions for definitive structural studies**

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*In vitro* prion propagation has been one of the greatest advances in the research field of Transmissible Spongiform Encephalopathies (TSEs). The use of bacterial recombinant prion proteins in the diverse cell-free prion propagation systems, besides definitively demonstrating the protein-only hypothesis, constitutes a highly versatile tool to study molecular mechanisms of the prion misfolding event. However, in order to completely understand the biology of prions, it is essential to decipher the three-dimensional structure of different prion strains at an atomic level resolution. Unfortunately, this is hindered by some requirements of the most suitable technique for amyloid structure resolution, solid state Nuclear Magnetic Resonance (ssNMR), such as the need of significant homogeneity of the sample under study and large amounts of isotopically labelled material. Although the Protein Misfolding Cyclic Amplification (PMCA) technique is widely used for the study of molecular mechanisms of prion misfolding, its scalability is limited by the sonication equipment required. Here, we present a new technique for infectious recombinant prion propagation *in vitro* that easily overcomes the isotopically labelled sample amount limitation for ssNMR studies. The procedure is based on PMCA, from where the misfolded recombinant seed is obtained. This highly infectious seed has been adapted to a new system in which shaking is used instead of sonication for recombinant prion propagation *in vitro*. Therefore, this novel propagation method has been named Protein Misfolding Shaking Amplification (PMSA). This methodology eliminates the need of complex and expensive equipment permitting its implementation in any basic laboratory. Thus, an affordable, easy, do-it-yourself, and highly scalable method is detailed, that allows the generation of more than 2 mg of infectious recombinant prion per day ready to be studied (after protease K treatment). The optimization applied to PMSA permits more than 1000-fold scaling up from minute amounts of seed, of isotopically labelled material suitable for structural studies. Moreover, PMSA offers other advantages over PMCA, as the possibility to implement a high-throughput screening system of

anti-prion compounds targeted to inhibit misfolding and propagation of prions. Therefore, a novel and highly versatile tool is presented herein for infectious recombinant prion propagation *in vitro*, easily applicable in any laboratory.

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## **O25    Rationally designed, structure-based vaccine candidates targeting Chronic Wasting Disease**

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Chronic wasting disease (CWD) is the most contagious prion disease, affecting free-ranging and captive cervids, including deer, elk, and moose. Like all prion diseases, CWD occurs due to misfolding of the cellular prion protein (PrP<sup>C</sup>) into the infectious conformer (PrP<sup>Sc</sup>), which is prone to aggregate into amyloid fibrils. No cures or prophylactic vaccines exist for any prion disease including CWD, resulting in an invariably fatal outcome for an infected host.

Although the high-resolution structure of PrP<sup>Sc</sup> has eluded experimental determination due to its insolubility and propensity to aggregate, the repeating nature of PrP<sup>Sc</sup> aggregates (e.g. amyloid fibrils) has allowed the collection of lower-resolution structural data via X-ray fiber diffraction, cryo-electron microscopy, and other techniques, all of which indicated a four-rung  $\beta$ -solenoid architecture for PrP<sup>Sc</sup>. The structure of the prion domain of the fungal HET-s prion protein was determined by solid-state NMR spectroscopy to contain a two-rung  $\beta$ -solenoid structure. This structural similarity made the HET-s prion domain an ideal target to create a conformational mimic for the structure of PrP<sup>Sc</sup>. By doubling the coding region of the HET-s prion domain, connected by a flexible linker, a four-rung  $\beta$ -solenoid variant was engineered, termed HET-2s.

The similarity of the  $\beta$ -solenoid fold allowed us to use HET-2s as an innocuous scaffold for the rational design of structure-based vaccine candidates targeting CWD. The alternating position of amino acid side chains within the  $\beta$ -strands (inwards vs outwards facing) was used to strategically place predicted surface residues of PrP<sup>Sc</sup> onto the surface of HET-2s. Constructs were expressed in *E. coli*, purified, and assembled into amyloid fibrils morphologically indistinguishable from native HET-s fibrils. Vaccine candidates showing good fibrillization, and therefore adopting the proper  $\beta$ -solenoid fold, were used to immunize both Prnp<sup>-/-</sup> and FVB wild type mice. The resulting anti-serum was titrated against the original antigen, before being tested for specificity against PrP<sup>Sc</sup>. One of our constructs, 14R1, containing just seven PrP-based residues from before the hydrophobic region and the first  $\alpha$ -helix, resulted in an immune response that recognized CWD brain homogenate, but did not bind uninfected brain homogenate.

We are currently testing the anti-serum against other prion diseases, since the predicted PrP<sup>Sc</sup> surface residues may also be exposed in other prion strains. The efficacy of the vaccine candidates will be tested in oral prion-infection models. Moreover, we are trying to establish clones expressing PrP<sup>Sc</sup>-specific monoclonal antibodies from the spleens of the immunized mice. Lastly, it has not escaped our attention that this approach of engineering the surface of an innocuous amyloid scaffold could be used to generate immunological mimics (i.e. vaccine candidates) targeting more common neurodegenerative diseases such as Alzheimer's or Parkinson's disease.

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## O26 Serpina3n plays a critical role in prion infection

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Keywords: serpin3, serpin3n, prion

**Background:** In a previous work, we identified a five-gene signature able to distinguish intracranially BSE-infected macaques from healthy ones, with *SERPINA3* showing the most prominent dysregulation. More recently, we showed that this striking up-regulation, both at the mRNA and at the protein level, is evident since the pre-clinical stage in RML-infected mice and post-mortem in virtually all forms of human prion diseases. In the current study, we analyzed in more details the correlation between Serpina3n –the mouse orthologue- expression and prion accumulation. In particular, we investigated the effects of the over-expression and of the ablation of the protein – either genetically or pharmacologically.

**Methods:** We first performed stable plasmid transfection for overexpression or knock-out of Serpina3n in GT1, N2a and ScN2a cell lines. *De novo* infection was then achieved by incubating both parental and transgenic GT1 and N2a cell lines with a solution of sonicated chronically infected ScGT1 or ScN2a for 7-8 days. After a week, the medium was refreshed and cells were passaged for 6-7 weeks before checking PrP<sup>Sc</sup> presence. Moreover, *in silico* screening of potential Serpina3n inhibitors was performed and the hit compounds were first tested *in vitro* for their toxicity and then for their efficacy in reducing prion accumulation.

**Results:** We observed that overexpression of Serpina3n in N2a cell line led to a faster PrP<sup>Sc</sup> accumulation and to an increased load of prions overall (about 4-fold) upon *de novo* infection. On the other hand, *de novo* infection of Serpina3n KO GT1 cell line showed a slower PrP<sup>Sc</sup> accumulation and a reduced prion load (about 60% less) over time. In addition, ablation of Serpina3n in chronically infected ScN2a cell line showed highly reduced or not detectable PrP<sup>Sc</sup> levels after few passages in culture. Similarly, treatment of ScGT1 and ScN2a with 20 μM Serpina3n inhibitors for 4 days strongly reduced the accumulation of PrP<sup>Sc</sup> (60-70% less).

**Conclusions:** We identified a protein – serpin3- which is strongly up-regulated both at the mRNA and protein level in prion infected cell lines, mice, macaques and humans. In particular, we showed that this up-regulation is evident since the pre-clinical stage in mice and also in *de novo* infected cell lines, suggesting a role for serpin3n not only in supporting the progression of the disease but also in the onset. More importantly, we observed that ablation of the protein -or inhibition of its activity- strongly reduces the accumulation of PrP<sup>Sc</sup>, representing thus a powerful target for anti-prion drugs.

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## AI-APRI Workshop: Structural biology of prions

### WS1 Structural aspects of prion protein conversion to the infectious form

Witold Surewicz

Case Western Reserve University, Cleveland, USA

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**WS2 Profound seeding selectivities of tau aggregates associated with Alzheimer's disease and other tauopathies**

Byron Caughey

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**WS3 Strain-specific PrP<sup>Sc</sup> structures and N-linked glycans**

Ilia Baskakov

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**WS4 High-resolution structures from A $\beta$ (1-42),  $\alpha$ -synuclein, PHF-tau, and HET-s(218-289) - why not from PrP<sup>Sc</sup>?**

Holger Wille

University of Alberta, Edmonton, Canada.

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**WS5 High resolution fibril structure of amyloid-  $\beta$ (1-42) by cryoelectron microscopy**

Dieter Willbold (1,2) and others

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We present the structure of an A $\beta$ (1-42) fibril composed of two intertwined protofilaments determined by cryoelectron microscopy (cryo-EM) to 4.0-Ångstrom resolution, complemented by solid-state nuclear magnetic resonance experiments. The backbone of all 42 residues and nearly all side chains are well resolved in the EM density map, including the entire N terminus, which is part of the cross- $\beta$  structure resulting in an overall "LS"-shaped topology of individual subunits. The dimer interface protects the hydrophobic C termini from the solvent. The characteristic staggering of the nonplanar subunits results in markedly different fibril ends, termed "groove" and "ridge," leading to different binding pathways on both fibril ends, which has implications for fibril growth. Each monomer that binds to a certain fibril end sees the same interface, in contrast to a true dimeric interface (in the case of a C2 symmetry), where added

monomers would alternately see either two identical binding sites or a cleft preformed by the preceding subunit. The  $\beta$  strands are staggered with relation to one another in a zipper-like manner. At both fibril ends, the binding site for the addition of subunit  $i$  contains contributions of subunits  $i-1$ ,  $i-2$ ,  $i-3$ ,  $i-4$ , and  $i-5$ , or  $i+1$ ,  $i+2$ ,  $i+3$ ,  $i+4$ , and  $i+5$ , respectively. Therefore, five A $\beta$ (1-42) subunits are required to provide the full interface for monomer addition. For a fragment of six subunits, the capping subunits would have the same full contact interface as those in an extended fibril. We define this structural element of six subunits as the minimal fibril unit. This minimal fibril unit may also be the minimal seed size for nucleation as suggested by results from A $\beta$  aggregation studies monitored by small angle neutron scattering and analytical ultracentrifugation.

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#### **WS6 Five Prion-curing systems in *Saccharomyces cerevisiae*: Upf proteins, inositol polyphosphates, Btn2p, Cur1p, and Hsp104p**

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The yeast prions [PSI<sup>+</sup>] and [URE3] are pathogenic amyloids of Sup35p and Ure2p, respectively, with the same in-register parallel  $\beta$ -sheet architecture as has been recently reported for two different infectious amyloids of PrP. We have found that five seemingly different yeast systems can cure one or the other of these prions at normal levels of the proteins. Each of these systems is eliminating most prions as they arise, although, like DNA-repair systems, none of these systems is perfect. a) Upf proteins carry out the nonsense-mediated mRNA decay process and are normally complexed with Sup35p, thereby preventing propagation of most [PSI<sup>+</sup>] variants formed in their absence. This suggests that this normal interaction can block the abnormal interactions that constitute prion formation. b) Some inositol polyphosphates (such as 5PP-IP<sub>5</sub> or IP<sub>6</sub>) can promote [PSI<sup>+</sup>] propagation, while 1PP-IP<sub>5</sub> blocks [PSI<sup>+</sup>] propagation. c) Btn2p collects Ure2p amyloid filaments (the [URE3] prion) at a single site in the cell, resulting in high frequency loss of the prion. d) Cur1p also specifically cures the [URE3] prion by a different mechanism. e) Hsp104p, with Hsp70s and Hsp40s, cleaves prion amyloid filaments, a process that is necessary for propagation of all of the yeast amyloid-based prions. Hsp104 overproduction is also well-known to cure [PSI<sup>+</sup>] by a distinct activity. We find that this second Hsp104 activity cures most [PSI<sup>+</sup>] variants arising in its absence. Thus, yeast has an array of anti-prion systems that dramatically reduce the prion load on the cell.

In contrast to the above systems that cure prions, the Ylr352w / Lug1 (lets [URE3] grow) protein does not cure [URE3], but is necessary for the growth of [URE3] strains on non-fermentable carbon sources. Lug1p is an F-box protein, a substrate-specifying subunit of a ubiquitinylation enzyme complex.

**See also poster P37**

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#### **WS7 Prion protein conformational landscape studied by mass spectrometry and ion mobility**

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Mass spectrometry has proved to be an efficient way to analyze “native” complexes with the view to bring valuable stoichiometric, and shape information with high sensitivity. More recently, the commercial availability of instruments coupled to ion-mobility devices has led to renewed interest of structural biologists with access to both conformational landscape and dynamic of proteins. Recent studies suggest that prion protein can follow a plurality of conversion pathways which hints towards different conformers that might coexist in solution. We therefore decided to screen the ovine and human PrP monomers using ion mobility coupled to mass spectrometry following electrospray ionization. After a short presentation of ion mobility for studying ionized proteins in the gas phase, we will briefly discuss issues with the collision cross section calibration procedure that we have encountered when using travelling wave ion mobility. We will also discuss the development of an automated data extraction pipeline for which we developed a Python/Qt script base interface. Infusion of monomeric PrP solutions have shown that at least three PrP conformers are observed in the gas phase. PrP monomers are known to lead to the formation of oligomeric species in specific conditions (temperature, pH and buffer), which are not compatible with mass spectrometry. We have therefore developed a size-exclusion chromatography IMS-MS setup with the aim to study the oligomers produced in these conditions. The development of this SEC-IMS-MS methodology will be presented as well as its application for calibration with standard protein complexes. Although we did not achieve resolution of the large (O1 ~36-mer) oligomeric species, optimization of the experimental parameters led to the observation of the small (O3) oligomeric species. One key observation in this process was that the abundance of the gas phase monomeric conformers changed upon SEC elution. We therefore envisioned that conformer family abundances were related to protein concentration. Thus we probed the effect of protein concentration upon conformer relative abundances. Results allowed us to clearly demonstrate that monomer concentration has an effect on the ratio of conformers present in solution, which could be related to the occurrence of structural information transfer between discreet polymeric objects.

**See also poster P43**

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## **WS8 Prion:lipid analysis using membrane nanodiscs bounded by amphipathic polymers**

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Understanding the structure of native states of infectious prion proteins (PrP<sup>Sc</sup>) and their arrangement within the native lipid bilayer has been a daunting task. The challenge arises largely due to the extensive use of conventional detergents such as sarkosyl as well as phosphotungstic acid (PTA) derivatives during the purification of PrP<sup>Sc</sup>, which in turn can exclude physiological lipids and induces fibrillization of PrP<sup>Sc</sup> into amyloid. Hence, studies based on conventional PrP<sup>Sc</sup> preparations can result in a distorted representation of the in vivo PrP<sup>Sc</sup>-lipid interactions. We demonstrate here that a novel and fast method using a number of different styrene-maleic acid (SMA) copolymers can be employed without any conventional detergents to isolate and purify PrP<sup>Sc</sup> from Syrian hamster and RML mice brains infected with the Hyper prion strain. Western blots and silver stained SDS-PAGE gels of the samples, which are highly enriched in lipids, confirm the presence of PrP<sup>Sc</sup> in different glycosylation states. The samples were further analyzed by negative stain electron microscopy highlighting key features of the detergent-free purified

PrP<sup>Sc</sup> including relatively ordered arrays of lipid-PrP<sup>Sc</sup> fibrils, formation of 2D crystals under certain experimental conditions and lipid:protein ratios and the appearance of the PrP<sup>Sc</sup> fibrils similar to those of detergent purified samples. The SMA platform promises new avenues towards an in-depth structural, biochemical, and pathological characterization of PrP<sup>Sc</sup> and its assortment of strains.

See also poster P4

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**WS9 Differences in the folding dynamics of prion proteins from species with different disease susceptibility observed at the single-molecule level**

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PrP is highly conserved across the animal kingdom, yet disease susceptibility varies widely, with some species being highly susceptible whereas others are resistant to varying degrees. These species differences appear to be driven by sequence differences in just a few amino acids, but how these changes alter the misfolding dynamics remains unclear. We explored the effects of species-related sequence differences on PrP folding by using force spectroscopy to observe the folding dynamics of single molecules held in optical tweezers. We compared the behaviour of PrP from hamsters (HaPrP) and bank voles (BvPrP) to that of PrP from rabbits (RaPrP) and dogs (CaPrP): hamsters are disease-susceptible, rabbits are quite resistant, dogs appear to be immune, and bank voles are amongst the most susceptible of all species. Unfolding and refolding trajectories were measured while ramping the force applied by the tweezers up and down. The resulting force-extension curves (FECs) revealed the existence of any on-pathway intermediates or misfolded (off-pathway) states, reflecting also the energetics and kinetics of the different states. In contrast to HaPrP, which was found previously to exhibit two-state folding, the folding of RaPrP and CaPrP involved multiple on-pathway intermediates; in both cases, native folding was rapid and misfolded states were not detected in FECs. For BvPrP, FECs included zero or at most one on-pathway intermediate, but the folding kinetics were much slower. Notably, metastable misfolded states with lifetimes on the order of seconds were observed for BvPrP. Hysteresis between unfolding and refolding FECs was seen for both HaPrP and BvPrP but not RaPrP and CaPrP, suggesting that disease resistance is associated with smaller energy barriers. Finally, by relating the contour-length changes observed in the FECs to structural features of the proteins, possible intermediates in the native folding pathways for RaPrP, CaPrP, and BvPrP were deduced. These results show that the subtle sequence differences between PrP from different species produce significant differences in the folding dynamics.

See also poster P7

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**WS10 Full-length human prion protein displays a whole variety of conformational transitions as observed by high hydrostatic pressure (HHP), xenon as a probe and spin labelling of the N-terminal domain**

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Transmissible spongiform encephalopathies (TSEs) are a group of fatal neurodegenerative disorders associated with the accumulation of fibrils of misfolded prion protein PrP. Conformational intermediates of the human prion protein *hu*PrP<sup>c</sup> were characterized by a combination of either high hydrostatic pressure (up to 200 MPa) as well as xenon gas pressure and spin labelling experiments at positions 93 or 107 with two-dimensional [<sup>1</sup>H, <sup>15</sup>N]-HSQC NMR spectroscopy. Xenon binds to four transiently enlarged hydrophobic cavities located in the well-folded core of human PrP(23-230). A fifth xenon binding site is formed transiently by amino acids A120 to L125 of the N-terminal domain and by amino acids K185 to T193 of the core. A model including a dynamic opening and closing of the cavities allowed to derive the individual microscopic and macroscopic dissociation constants. A structural coupling between the N-terminal domain and the core domain can now be confirmed by high pressure NMR spectroscopy, xenon binding and spin labelling experiments.

**See also poster P11**

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#### **WS11 A bacterial prion-like protein as a synthetic model to study amyloid toxicity**

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Protein amyloids are central to human neurodegenerative diseases. However, the mechanisms for amyloid cytotoxicity are still not fully defined. Bacteria have functional extracellular amyloids but no natural amyloid proteinopathy had been found yet.

The WH1 domain of the bacterial protein RepA can be engineered to assemble as amyloid fibres upon binding to specific DNA sequences. RepA-WH1 amyloid fibres are bundles made of intertwined tubular protofilaments, which are made of structurally distorted protein monomers (1,2). RepA-WH1 causes in *E. coli* an amyloid proteinopathy (3), which is *vertically* transmissible from mother to daughter cells, templating its conformation by cross-seeding *in vitro* and *in vivo*. However, RepA-WH1 is not infectious, i.e. is a 'prionoid'. Bacterial lineages maintain two mutually exclusive strains of RepA-WH1 amyloids: either multiple globular particles that inhibit cell division, or a single elongated aggregate, mildly detrimental to growth. The bacterial Hsp70 chaperone DnaK modulates the phase transition between both amyloid strains (4). Amyloidogenic oligomeric precursors of RepA-WH1 locate at the bacterial nucleoid (5).

Concerning its mechanisms of cytotoxicity, RepA-WH1 builds pores in lipid vesicles analogous to the *E. coli* inner membrane, leading to leakage of their contents (6). Systems Biology approaches show that RepA-WH1 amyloidosis in bacteria also implies the generation of ROS and the co-aggregation with the protein of essential cellular factors involved in the response to oxidative stress (7). This scenario of three concurrent cytotoxic events has counterparts in human amyloidoses. RepA-WH1 provides a unique window to survey the minimal landscape of an amyloid proteinopathy, endorsing this prion-like protein as a generic bacterial model for amyloid diseases (8).

(1) *Proc Natl Acad Sci USA* 104: 17388-93 (2007).

(2) *Structure* 23: 183-9 (2015).

(3) *Mol Microbiol* 77: 1456-69 (2010).

(4) *Mol Microbiol* 91: 1070-87 (2014).

(5) *Sci Rep* 5: 14669 (2015).

(6) *Sci Rep* 6: 23144 (2016).

(7) *Front Microbiol* 8: 539 (2017).

(8) *Prion* 10: 41-9 (2016).

**See also poster P12**

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## **WS12 Dynamics of prion replication and structural diversification**

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Prion, Alzheimer's and Parkinson's diseases share common pathogenic mechanisms. In principle, host-encoded proteins are converted into misfolded, aggregated assemblies, which serve as template for further autocatalytic conversion. In prion-infected species, host-PrP<sup>C</sup> is converted into the beta-sheet rich PrP<sup>Sc</sup> conformer. PrP<sup>Sc</sup> assemblies can form structurally distinct PrP<sup>Sc</sup> conformers or strains, encoding specific biological phenotypes. A large body of evidence support the view for further structural heterogeneity within prion strains, with respect to PrP<sup>Sc</sup> structure, biochemical properties and replicating activity.

The kinetic aspects of prion replication in the brain has served to elaborate theoretical and mathematical models for prion replication. The most accepted models merely consider prion as single/ homogeneous PrP entity all along the disease progress. The coexistence of structurally distinct PrP<sup>Sc</sup> assemblies within the same environment challenges these best-replicator selection models and questions how structurally diverse assemblies can coexist when they are in competition for the same substrate, i.e. PrP<sup>C</sup>.

Here, we addressed the question of prion replication and structural diversification during cell-free prion replication by protein misfolding cyclic amplification (PMCA), which mimics *in vivo* replication with accelerated kinetics (1). We analysed the dynamics of PrP<sup>Sc</sup> assembling/disassembling during the PMCA process by sedimentation-velocity-based methods (2). We show that: i) during the early step of prion amplification, two structurally distinct and discrete populations of PrP<sup>Sc</sup> assemblies are generated (namely pA<sub>1</sub> and pA<sub>2</sub>). pA<sub>1</sub> is preferentially generated and is of small size whereas pA<sub>2</sub> is a minor subcomponent of larger size. ii) pA<sub>1</sub> is likely to be generated from the dilution-induced disassembling of PrP<sup>Sc</sup> toward its elementary brick suPrP (3), and not from fragmentation of inoculum PrP<sup>Sc</sup> or from preferential amplification of the seeds with the highest templating activity, iii) pA<sub>2</sub> formation results from

further incorporation of PrP<sup>C</sup> by pA<sub>1</sub> through a highly cooperative process suggestive of a catalytic conversion of pA<sub>1</sub> to pA<sub>2</sub>, iv) Different prion strains (127S scrapie prions, 139A mouse prions, vCJD human prions) exhibit similar replication dynamic, suggesting a rather generic process.

Altogether these observations suggest an intrinsic propensity of prion replication process to generate structurally distinct assemblies even in highly controlled media such as PMCA conditions. The conversion of pA<sub>1</sub> into pA<sub>2</sub> assemblies in the presence of PrP<sup>C</sup> reveals the highly dynamic character of PrP<sup>Sc</sup> assemblies.

1. Moudjou *et al.* *MBio* **5**, e00829-00813 (2014).
2. Tixador *et al.* *PLoS Pathog* **6**, e1000859 (2010).
3. Igel-Egalon *et al.* *PLoS Pathog* **13**, e1006557 (2017).

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### **WS13 A New Structural Model of PrP<sup>Sc</sup> Based on Recent Experimental Data and Computational Techniques.**

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While elucidation of the structure of PrP<sup>Sc</sup> has proven to be a phenomenal experimental challenge due to its high insolubility and aggregation propensity, recent experimental data derived from Cryo-Electron Microscopy and fiber X-ray diffraction studies coincide to show that its architecture conforms to a 4-rung  $\beta$ -solenoid [1]. Furthermore, a recent re-evaluation of early FTIR data [2] supports the conclusion that PrP<sup>Sc</sup> does not contain any substantial amount of  $\alpha$ -helical secondary structure.

Armed with this knowledge, we have used computational tools to construct a realistic full-atomistic model of PrP<sup>Sc</sup>. Our approach consists in threading the PrP sequence into the scaffold of typical  $\beta$ -solenoid proteins, adapting the structures thus obtained and testing their stability using molecular dynamics simulations.

We have proceeded in three steps: first, we developed a Python program able to predict which of the PrP residues belongs to a particular secondary structure (eg:  $\beta$ -strand or coil) on the target conformation. This process considers each amino acid structural propensity, also taking into account limited proteolysis data [3] that provide circumstantial evidence of where some of the loops connecting the  $\beta$ -strands might be localized. Then, we applied different modelling tools to generate and optimize 3D atomistic structures based on our predictions. Finally, we adapted and tested the structures thus obtained using molecular dynamics simulations.

With this approach we have constructed a physically stable PrP<sup>Sc</sup> monomer model satisfying all the available experimental data. The model maintained a substantial structural stability over three preliminary 50 ns simulations, at the end of which 95% of the initial  $\beta$ -sheet secondary structure skeleton stayed intact and a negligible overall structural deviation (RMSD) from the proposed model of 0.45 nm (including the loops) was observed. More extended simulations are in course and we expect that they will confirm extended stability. Furthermore, 3D models of a PrP<sup>Sc</sup> oligomer, based on stacking of PrP<sup>Sc</sup> monomers, are also under development and stability testing. Based on theoretical considerations, for example, further stabilization of edge  $\beta$ -strands through intermolecular hydrogen bonding, increased stability is expected.

While it is obvious that our model and PrP<sup>Sc</sup> might diverge in many aspects, our proposed structure constitutes a proof of principle of the stability of a 4-rung solenoid conformation of the PrP sequence, illuminates the mechanism of PrP<sup>Sc</sup> propagation and offers an atomistic workbench to test hypotheses to understand and explain the transmission barrier and strain phenomena.

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**See also poster P21**

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**WS14 Effects of mutations, polymorphisms, and other variants of the PRND gene on the PrP<sup>C</sup> structure from MD simulations analyzed with essential collective dynamics and machine learning methods**

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Misfolding of prion proteins (PrPs) can cause severe neurodegenerative disorders of infectious, inherited, or sporadic nature, such as Gerstmann-Sträussler-Scheinker syndrome (GSS), Creutzfeldt-Jakob disease (CJD), fatal familial insomnia (FFI), and Kuru [1]. However, the detailed structure of the misfolded infectious isoform, PrP<sup>Sc</sup>, and the pathways of the conversion from native PrP<sup>C</sup> to PrP<sup>Sc</sup> remain largely unknown. Inherited prion diseases have been associated with point mutations in the gene encoding PrP. In this study, we have investigated models of human PrP90-231 for the wild type (WT) allele and 113 point mutations and insertions. Some of these mutations are linked to familial prion diseases [2], others are considered protective or neutral [2], while others were found as missense variants in genotyped individuals [3].

An NMR-derived structure of WT PrP<sup>C</sup> was used to model 113 variants. All-atom molecular dynamics simulations were performed on these systems [4]. Structural and dynamic effects of the mutations were analyzed using a suite of descriptors such as RMSD, changes in secondary structure, inter-residue distances, and solvent accessible surfaces. A novel essential collective dynamics (ECD) tool [5] was employed extensively. The changes in structure and dynamics specific to each mutation were thoroughly compared across all the systems, especially with respect to WT PrP and known protective polymorphisms. The data were also processed with machine learning algorithms.

Regions where observed transformations in secondary structure were the most pronounced include loop LH2H3 and  $\alpha$ -helix H3. Notable differences between protective polymorphisms and known disease-causing mutations were also found for the solvent accessible surface area of a hydrophobic pocket (HP) between H2H3 and S1S2 bundles, as well as other characteristics relevant to the HP's exposure or propensity to open, such as distance between residues M166 and

Y218. The ECD main-chain flexibility of loops LS2H2 and LH2H3 also was found to be important.

Subgroups of mutations were identified based on observed differences in several descriptors. Among numerous structural and dynamical descriptors investigated, no single descriptor alone would represent all known pathogenic or protective properties of the studied amino acid substitutions. Combining different descriptors that reflect complementary aspects of the structure and dynamics of the huPrP globular domain was found to be crucial for identifying fingerprints of non-pathogenicity of the variants.

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**See also poster P30**

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## **WS15 Properties of Prion Self-replication**

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Prion diseases is a group of fatal neurodegenerative disorders that affect many mammalian species. In the most of cases occurrence of these diseases in humans is spontaneous or is a result of mutations in the PrP gene PRNP, however, in a relatively rare cases it might be an infection due to exposure to prions via medical procedures, prion-contaminated food or cannibalism. The main causative agent of prion diseases is thought to be the aggregated form of native prion protein (PrP<sup>C</sup>), known as PrP<sup>Sc</sup>. The infectivity of prion diseases is based on PrP<sup>Sc</sup> ability to self-propagate by binding to PrP<sup>C</sup> and inducing its conformational conversion to the PrP<sup>Sc</sup> state. One of the most interesting aspects of prion diseases is existence of multiple “strains”, which lead to different disease phenotypes that are distinguished by distinct clinical signs, incubation time and neuropathology. Formation of distinct self-replicating states of PrP<sup>Sc</sup> was reported in multiple *in vivo* and *in vitro* experiments, while some of these states are able to self-replicate only under certain conditions *in vitro*, others are capable of replicating under broad range of conditions *in vitro* and *in vivo*. To date there is still no clear understanding what leads to formation of wide variety of strains, or how many different strains can PrP form.

The aim of this study is to determine and compare the effect of temperature and denaturant concentration on elongation kinetics of distinct mouse prion fibril self-replicating states. Results of this research could contribute to the deeper understanding of amyloid polymorphism and self-propagation.

Distinct self-replicating states of mouse prion protein fibrils display different profiles of elongation kinetics under various environmental conditions. In some cases, unusual profile of elongation kinetics point to possible remodeling of existent amyloid fibrils or presence of multiple

polymorphs in a sample, however, due to complexity of the aggregation process it is difficult to determine the real cause and deeper investigation is needed.

**See also poster P3**

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**WS16 A new solid-state NMR approach to determine 3D structures of prion amyloid fibrils at atomic resolution**

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The amyloid fold is a protein fold involved in different neurodegenerative diseases and in prion propagation mechanisms. In addition, several functional amyloids have also been identified in bacteria, fungi and mammals. Thus numerous proteins can adopt this type of fold, but at the same time amyloid propensity is also highly sequence dependent. The low number of amyloid atomic structures has limited the exploration of the sequence-to-structure interplay for this important class of protein fold.

We develop a solid-state NMR-based approach (1) for atomic structure determination in the fibrillar propagative state to establish amyloid sequence-to-fold relation. Our approach aims at determining prion amyloid fibril structure at an unprecedented atomic resolution. Notably, the quality of solid-state NMR data obtained here compares favorably with the structure determination of the HET-s prion domain (2), the current benchmark in amyloid fibril structure determination.

Using this approach, we compare atomic structures of two highly divergent paralogous functional fungal prions involved in signal transduction cascades. We report a remarkable conservation of their fold, despite extreme sequence divergence. While identical backbone structures occur in these prions despite a very low level of sequence similarity, lack of cross-seeding suggests that a common amyloid fold is not sufficient to permit amyloid templating.

(1) Saupe, Habenstein, Loquet and co. to be submitted

(2) Wasmer et al., Science 2008.

**See also poster P4B**

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**WS17 DNP-Enhanced solid-state NMR at Cryogenic Temperatures: a Tool to Snapshot Conformational Ensembles of  $\alpha$ -Synuclein in Different States**



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Dynamic Nuclear Polarization (DNP) overcomes the inherently low sensitivity of magnetic resonance methods by transferring high spin polarization of unpaired electrons to surrounding nuclei. Low-temperature Nuclear Magnetic Resonance (NMR) spectra usually suffer from severe line broadenings due to freezing out different conformations<sup>1</sup>. While this is usually accounted for as an unwanted side-effect of DNP-NMR, these inhomogeneously broadened lines also contain valuable information about conformational ensembles of (disordered) proteins.

To study conformational ensembles of intrinsically disordered proteins, both experimental and computational methods have evolved. For the experiments reported here, we have chosen  $\alpha$ -synuclein ( $\alpha$ -syn) as a model protein, and the large-scale conformational flexibility is investigated by DNP-enhanced NMR and molecular dynamics (MD) simulation. We have studied the conformational ensemble of  $\alpha$ -syn in frozen solution under different conditions: in the fully disordered form, in the fibrillated form with flexible ends, and in contact with lipid bilayers in the form of nanodiscs (in different protein/nanodisc ratios). We could probe the conformational ensembles of all valine residues in a selectively labeled sample of  $\alpha$ -syn<sup>2</sup> by evaluating the inhomogeneously broadened line-shape of the  $C\alpha/C\beta$  cross peak. For the free disordered monomer in frozen solution, two maxima are observed, one located in the typical  $\alpha$ -helical chemical shift region, and the other in the typical  $\beta$ -strand chemical shift region<sup>3</sup>. Based on these results, we could estimate the amount of disordered regions in fibrillar  $\alpha$ -syn and estimate membrane binding regions of  $\alpha$ -syn bound to membranes in different protein to lipid ratios. Furthermore, secondary chemical shifts of neighboring amino acids tend to be correlated, indicative of formation of transient secondary structure elements. Our approach thus provides accurate quantitative information on the propensity to sample transient secondary structures in different functional states which is also in line with our previously performed solution NMR titration experiments and MD simulations<sup>4</sup>.

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(2) Hong M, Jakes K. Selective and extensive <sup>13</sup>C labeling of a membrane protein for solid-state NMR investigations. J Biomol NMR 1999, 14, 71-4.

(3) Uluca et al, DNP-Enhanced solid-state NMR at Cryogenic Temperatures: a Tool to Snapshot Conformational Ensembles of  $\alpha$ -Synuclein in Different States. Biophys. J. 2018, in press. doi:10.1016/j.bpj.2018.02.011

(4) Viennet et al, A structural and kinetic link between membrane association and amyloid fibril formation of  $\alpha$ -Synuclein. Communications Biology, 2018, accepted.

**See also poster P38**

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**WS18 Molecular basis for seeding barriers between three- and four-repeat tau**

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Neurofibrillary tangles composed of the microtubule associated protein tau are the pathological hallmark of Alzheimer's disease (AD), progressive supranuclear palsy (PSP), and other fatal neurodegenerative disorders. Short tau fibrils are believed to spread intracerebrally by transfer between interconnected neurons. Once taken up by the cell, tau fibrils recruit soluble tau monomers onto their ends and imprint their conformation onto the recruited proteins. Based on the number of microtubule binding repeats, there are two distinct groups of tau isoforms: three-repeat (3R) tau and four-repeat (4R) tau. In AD all tau isoforms are recruited into fibrils. In PSP only 4R tau is deposited. Although it is recognized that the conformations of fibrils in PSP may be incompatible with the recruitment of 3R tau, the molecular events leading to this barrier are not understood. We hypothesized that proteolytic cleavage of 4R tau prior to fibrillization could be a contributing factor. To test this hypothesis we formed tau fibrils from an array of differently sized 4R tau fragments. We identified a broad spectrum of barriers in 4R tau truncated at the N- and C-termini. A fragment of the repeat region (amino acids 244-368) established the strongest barrier. Remarkably, single mutations in full-length tau elicited similar effects. Collectively, the findings reveal sequence-dependent variations in tau fibril structure that determine seeding barriers in 4R tau.

See also poster P117

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#### **WS19 Heparan sulfate modulates the brain distribution of fibrillar prions**

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A major unresolved question in neurodegenerative diseases is the mechanism underlying selective cell vulnerability. Heparan sulfate proteoglycans (HSPGs) are ubiquitously expressed, heterogeneous glycoproteins that accumulate in prion amyloid plaques in the extracellular matrix. HSPGs also bind prions on the cell surface promoting their internalization in neurons. To address the hypothesis that prion interaction with HS is a major determinant underlying selective cell vulnerability, we investigated how alterations in HS impact the propagation and distribution of prions *in vivo*. Three mouse-adapted prion strains were inoculated intracerebrally into transgenic mice expressing altered HS chains as well as wild type littermate controls. We found that mutant mice showed a delayed disease course and a shift in the brain plaque distribution when exposed to a fibril-forming strain, but showed no differences in the disease phenotype when exposed to subfibrillar prions. There were no differences in the inflammatory response to the prions, nor any alteration in the prion conformation, as the electrophoretic mobility, glycoprofile, and stability of misfolded PrP (PrP<sup>Sc</sup>) were nearly identical to the controls. Additionally, there were no major differences in heparin as a cofactor promoting the *in vitro* replication of subfibrillar and fibrillar prions in protein misfolding cyclic amplification experiments. Importantly, we found that the fibrillar prion is predominantly formed by cleaved PrP<sup>Sc</sup>, lacking the glycoposphatidylinositol anchor, which explains its extracellular accumulation and binding to HS. Altered HS chains may result in a lower binding affinity of extracellular prions to HS and a redistribution of the prion plaques. Our results suggest that HS together with the prion post-translational modifications,

endoproteolytic cleavage, and aggregate conformation determine the binding affinity of prions to HS and underlie the specific regional targeting in the central nervous system.

**See also P15**

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## **WS20 Recombinant PrP<sup>Sc</sup> as a surrogate of brain PrP<sup>Sc</sup> for structural studies**

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## **AI-APRI Workshop: Animal prion disease**

### **WA1 An overview—Chronic Wasting Disease mother to offspring transmission studies conducted at Colorado State University**

Nalls AV<sup>1‡</sup>, McNulty EE<sup>1‡</sup>, Hoover C<sup>1</sup>, Hoover EA<sup>1</sup>, Wild M<sup>2</sup>, Powers J<sup>2</sup>, Selariu A<sup>1</sup>, Mathiason CK<sup>1</sup>

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Chronic wasting disease (CWD) demonstrates remarkable transmission efficiency among captive and free-ranging cervid populations. Intrigued by this facile transmission, we designed a series of studies to determine the potential for CWD transmission from mother to offspring. Viable offspring born to early or late-stage CWD-infected Reeves' muntjac dams demonstrated lymphoid biopsy positivity as early as 40 days post birth and developed clinical disease in 2-5 years. Prion infectivity was validated in the milk and colostrum collected from these dams via oral infection studies in naïve fawns, providing a potential mechanism for maternal transmission. High nonviability (~60%) was noted in full-term offspring born to this cohort of CWD-infected muntjac dams. Tissues harvested from the nonviable offspring contained prion seeding activity, suggesting that CWD had been trafficked from mother-to- offspring during gestation. Gestational timing of CWD transfer was assessed by analysis of in utero harvested tissues from a second cohort of preclinical and clinical CWD-infected muntjac dams, and it was found that transmission can occur as early as the first trimester of pregnancy. The next series of studies were initiated to determine if this was a phenomenon of experimental infection, or whether it also occurs in naturally-infected cervids. In collaboration with the National Park Service we evaluated in utero derived tissues from healthy free-ranging elk dams whose habitat is a known CWD endemic region. Prion seeding activity was detected in fetal tissues harvested from several preclinical CWD positive naturally-infected dams in this population. To begin to unravel the biological relevance of these findings we initiated mouse bioassay to assess the infectivity of the pregnancy

microenvironment of muntjac dams. Uterus, placenta, ovary and amniotic fluid contained prion infectivity. Ongoing mouse bioassay will further define infectivity in fetal and reproductive tissues harvested from free-ranging naturally-exposed elk dams. This work has led to a better understanding of the transmission dynamics of CWD, demonstrating that: (i) fetuses of preclinical-infected cervid dams are exposed to CWD long before exposure to maternal saliva or contaminated environments, (ii) CWD mother-to-offspring transmission occurs in free-ranging naturally-exposed cervid populations, and (iii) the Reeves' muntjac deer can be used to further explore mechanisms of in utero prion trafficking and the potential for multigenerational CWD transmission.

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**WA2 Oral transmission of CWD into *Cynomolgus* macaques: signs of atypical disease, prion conversion and infectivity in macaques and bio-assayed transgenic mice**

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To date, BSE is the only example of interspecies transmission of an animal prion disease into humans. The potential zoonotic transmission of CWD is an alarming issue and was addressed by many groups using a variety of in vitro and in vivo experimental systems. Evidence from these studies indicated a substantial, if not absolute, species barrier, aligning with the absence of epidemiological evidence suggesting transmission into humans. Studies in non-human primates were not conclusive so far, with oral transmission into new-world monkeys and no transmission into old-world monkeys. Our consortium has challenged 18 *Cynomolgus* macaques with characterized CWD material, focusing on oral transmission with muscle tissue. Some macaques have orally received a total of 5 kg of muscle material over a period of 2 years. After 5-7 years of incubation time some animals showed clinical symptoms indicative of prion disease, and prion neuropathology and PrPSc deposition were found in spinal cord and brain of euthanized animals. PrPSc in immunoblot was weakly detected in some spinal cord materials and various tissues tested positive in RT-QuIC, including lymph node and spleen homogenates. To prove prion infectivity in the macaque tissues, we have intracerebrally inoculated 2 lines of transgenic mice, expressing either elk or human PrP. At least 3 TgElk mice, receiving tissues from 2 different macaques, showed clinical signs of a progressive prion disease and brains were positive in immunoblot and RT-QuIC. Tissues (brain, spinal cord and spleen) from these and pre-clinical mice are currently tested using various read-outs and by second passage in mice. Transgenic mice expressing human PrP were so far negative for clear clinical prion disease (some mice >300 days p.i.). In parallel, the same macaque materials are inoculated into bank voles. Taken together, there is strong evidence of transmissibility of CWD orally into macaques and from macaque tissues into transgenic mouse models, although with an incomplete attack rate. The clinical and pathological presentation in macaques was mostly atypical, with a strong emphasis on spinal cord pathology. Our ongoing studies will show whether the transmission of CWD into macaques and passage in transgenic mice represents a form of non-adaptive prion amplification, and whether macaque-adapted prions have the potential to infect mice expressing human PrP. The notion that CWD can

be transmitted orally into both new-world and old-world non-human primates asks for a careful reevaluation of the zoonotic risk of CWD.

**See also poster P103**

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### **WA3 How is the CWD situation in Norway?**

Benestad SL (1), Tran L (1), Saure B (1), Terland R (1), Svendsen S (1), Dessen K (1), Haugum M (1), Handeland K (1), Madslie K (1), Kolbjørnsen Ø (1), Wisløff H (1), Moldal T (1), Våge J (1), Pirisinu L (2), Vaccari G (2), Bian J (3), Moreno JA (3), Kim S (3), Spraker T (4), Telling GC (3) R, Nonno R (2), Vikøren T (1)

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Chronic wasting disease (CWD) affects cervids and belongs to the group of transmissible spongiform encephalopathies (TSE), also called prion diseases, together with scrapie in small ruminants, bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt-Jakob disease in humans.

CWD was first detected in North America (Colorado) 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 25 American states and two 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.

The clinical signs of CWD are subtle and vary from case to case. The first clinical signs are nonspecific and include variable behavioral changes such as listlessness, isolation from the herd, lowering of head and ears, repetitive walking and hyperexcitability, followed by weight loss. Other signs are polydipsia/polyuria, hypersalivation, grinding of the teeth, frequent regurgitation and difficulty swallowing. The duration of clinical disease varies among individuals, ranging from weeks to months. CWD is the most contagious of the prion diseases, transmitted by direct contact from deer to deer, or 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 first diagnosed 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 biochemical and IHC characteristics indistinguishable from those of the CWD cases identified in North America. The Norwegian Ministry of Agriculture and Food has put in action measures for eradication of CWD in the Nordfjella reindeer population. Culling and testing of the 2 200 local reindeer started in August 2017 and is now complete. Of 2471 reindeer tested in the Nordfjella area, 19 were diagnosed with CWD, indicating a prevalence under 1%.

Immediately after the first case of CWD in 2016, an enhanced surveillance program for CWD started in Norway; >41 300 cervids have been tested with 23 CWD cases were detected. In addition to the 19 positive reindeer, all from Nordfjella, three moose, and recently one red deer, have been identified as positive for PrP<sup>Sc</sup>. These four animals are old (13-16 years), were found in three regions located approximately 300 to 500 km away from Nordfjella, and have different biochemical and IHC characteristics from the North American CWD cases.

Preliminary bioassay results show that the reindeer and the moose prion type are distinct from each other's. In addition, they are also different from the North American CWD suggesting that different strains affect European cervids compared to North America. Further research is ongoing to characterize the disease, especially the red deer, which shows some peculiar biochemical and histopathological characteristics.

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#### **WA4 Evolution of Chronic Wasting Disease Prion Conformers**

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Chronic wasting disease (CWD) in cervids involves the misfolding of cellular prion proteins (PrP<sup>C</sup>) into infectious, strain-encoding protein structures (PrP<sup>CWD</sup>) termed prions. Conversion and recruitment of host PrP<sup>C</sup> into PrP<sup>CWD</sup> aggregates sustains replication. Transmission of CWD within and between cervid species expressing PrP<sup>C</sup> amino acid polymorphisms is thought to drive mutation and host-genotype dependent selection of strains with novel properties. We compared the biochemical, structural and transmission properties of deer CWD prions composed of different PrP primary structures. Cervid PrP<sup>C</sup> polymorphisms introduced conformational modifications that affected the levels of PrP<sup>CWD</sup> and the structural stability of PrP<sup>CWD</sup> aggregates. Other properties supporting this conclusion include: variable electrophoretic profile of PK-res PrP<sup>CWD</sup>, C-terminal PrP physiological cleavage and protease sensitivity. These conformational differences encode at least two distinct CWD strains that are differentially selected depending on the next host PrP<sup>C</sup>. Transmission of CWD prions in deer expressing allelic variants of PrP<sup>C</sup> drives prion strain conformational diversification in affected cervid populations. Emergent CWD strains can have altered host ranges, modifying transmission dynamics, disease detection and vaccine-based CWD eradication.

**See also poster P105**

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#### **WA5 Assessing CWD Prion Neuro-invasion by Longitudinal CSF Analysis in Deer**

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While the duration of CWD infection in cervids can last more than 30 months post exposure, the time that neuro-invasion occurs remains unknown. To elucidate this issue, we collected and

examined multiple cerebrospinal fluid (CSF) samples spanning a 6 month period, from 12 CWD-inoculated white-tailed deer involved in longitudinal CWD studies. To seek evidence of early neural invasion and prion amplification, CSF samples were assayed by standard and iron-oxide magnetic extraction real-time quaking induced conversion (IOME-RT-QuIC). We found that in 3 animals, RT-QuIC seeding activity in CSF was detectable at 6 months (n=1) and 9 months (n=2) after the first detection of PrPCWD in biopsied tonsil or peri-anal lymphoid tissues by immunohistochemistry (IHC). With 2 of the 3 deer, in which we were able to collect CSF samples 3 months after the first IHC positive result, prion seeding activity was not detected. By comparison, in 77 CWD-infected deer where CSF was collected at terminal stage disease, RT-QuIC seeding activity was detected in 51 (72%) samples with a range of 0 to 27 months [mean 13.8] after initial IHC positivity. We conclude that neuro-invasion and prion amplification in the brain of deer can occur between 3 and 6 months after an initial PrPCWD detection by IHC in lymphoid tissue.

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## **WA6 Advances in CWD prion research**

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An overarching, long-term goal of our research is to understand the parameters controlling prion transmission and evolution within and between species, and ultimately to prevent recurrences of prion epidemics among humans and animals. A specific focus of our program is chronic wasting disease (CWD), a burgeoning global epidemic of deer, elk and other cervids, of increasingly uncertain zoonotic potential. The only known prion disease of wild animals, its contagious spread in North American deer, elk, and moose appears irrevocable. Imported North American CWD spread from farmed to novel native species in South Korea, and more recently CWD was identified in wild Norwegian reindeer and moose. In March 2018, Finland also recorded a case of CWD in a wild moose. Here we review the results of our recent studies on CWD. This includes the development of prototype, CWD-susceptible transgenic (Tg) mice; their application to ascertain CWD strain prevalence, mechanisms of natural disease transmission, and zoonotic risk; Tg modelling of the effects of naturally occurring polymorphic variation in cervid PrP genes; cell-free and cell culture models to characterize CWD prions; and the recent development of gene-targeted (Gt) mouse models in which expression of cervid PrP faithfully recapitulates that of native host PrPC. We will also review our recent comparative analyses that reveal differences in the biological properties of prions causing CWD in North American and European cervids.

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## **WA7 Interaction of CWD Prions with Environmental Samples**

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The environmental persistence of CWD infectivity represents a long-term, intractable, environmental dilemma. CWD-infected cervids shed infectious agent into the environment from body fluids (saliva, feces and urine) and from diseased animal carcasses. Prion infectivity is resistant to degradation and can persist in the environment for years, binding to soils as well as

vegetation. The extreme diversity of soils and vegetation necessitates a comprehensive analysis of CWD prion interactions with environmental samples. Our studies contrast CWD prion interaction with soils and vegetation of prairie and boreal regions. Soil minerals differ considerably in their ability to bind prions. Montmorillonite, a clay common to CWD-endemic regions of North America, avidly binds CWD prions while prions do not adhere strongly to illite, a soil mineral present in boreal soils. Soil organic material can decrease PrP<sup>CWD</sup> levels. We find considerable prion infectivity washes off the majority of vegetation (grasses, tree leaves) while exhibiting greater adherence to lichens. These studies suggest a differing environmental impact of CWD between prairie and boreal ecosystems.

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#### **WA8 PMCA as a platform for pre-symptomatic CWD diagnosis. Validation in field samples**

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Chronic Wasting Disease (CWD) is a prion disease affecting a wide spectrum of cervids in both, captive and wild habitats. This disease has acquired notoriety due to their fast spread in North America and the recent identification of infectivity foci in Northern Europe. Unfortunately, there are not efficient diagnostic methods allowing to monitor and control the CWD spreading in risk areas. Currently, the most used diagnostic method for early CWD detection consist in the immunohistochemical analyses of *post-mortem* brain and lymphoid tissues. Although this test is normally accurate, it is highly invasive (mostly done *post-mortem*), expensive and time consuming. *In vitro* amplification methods, such as the Protein Misfolding Cyclic Amplification (PMCA) and real-time quaking induced conversion (RT-QuIC), have been proven as effective, fast and inexpensive means to detect misfolded prions in biological fluids of experimental and natural cases of prion diseases. Importantly, PMCA has been reported to detect minute amounts of infectious prions (PrP<sup>Sc</sup>) in a wide variety of biological samples, allowing for pre-symptomatic detection of prion disease with great sensitivity and specificity. In this work, we aimed to test the usefulness of PMCA as a platform for the pre-symptomatic diagnosis of CWD. In collaboration with the United States Department of Agriculture (USDA), we tested our *in vitro* prion amplification technology in several biological samples, including blood, feces, semen, and saliva collected from farms across the United States. Importantly, samples were collected from animals with poor body condition as well as from individuals at different stages of the pre-symptomatic incubation period (as tested for the *post-mortem* presence of PrP<sup>Sc</sup> in retropharyngeal lymph nodes and brain stem). Our results show that PMCA is able to detect the prion infectious agent in all biological matrices with specificities ranging from 95% to 100% depending on the sample (being the maximum specificity found in blood which is the cleaner specimen to collect in the field). Sensitivity varied, depending on the clinical stage (lower at early pre-symptomatic stages). We also considered the role of polymorphic changes at position 96 of the prion protein on PMCA performance using different versions of the cervid PrP as PMCA substrate. Our results show that PMCA is an efficient technique for the early detection of infectious prions in several biological samples. Our results may help to establish PMCA as a non-invasive and sensitive platform to



control for the presence of CWD cases. The PMCA assay may provide better monitoring practices to control the spread of this natural prion disease.

**See also poster P108**

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#### **WA9 Pig-PrP as a paradigm of resistance to prion propagation**

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Transmissible Spongiform Encephalopathy (TSE) has never been reported in pigs under natural conditions. However, pigs have been shown to be susceptible to classical Bovine Spongiform Encephalopathy (BSE) after experimental transmissions to pigs or transgenic mice expressing pig-PrP<sup>C</sup> (PoPrP-Tg001) although with a considerable transmission barrier.

In this work, the susceptibility of pigs to different prion strains is assessed in transgenic mice overexpressing pig-PrP<sup>C</sup> (PoPrP-Tg001). For that purpose, a broad panel of prion isolates representatives of different prion strains was intracerebrally inoculated in these mice. Different species (cattle, sheep, goat, mouse, hamster and human), PrP genotypes, geographical origin, biological properties and biochemical characteristics were taken into account for the selection of the inoculated strains. The panel of isolates tested included classical and atypical/Nor98 scrapie from sheep and goat, H- and L-type atypical BSE from cattle, rodent scrapie isolates (RML, 22L and 263K) and human Creutzfeldt-Jakob disease (CJD) prions. Furthermore, classical BSE isolates after passage in different species were also included.

The results showed that none of the PoPrP-Tg001 mice succumbed to disease after inoculation with any of the assayed classical or atypical scrapie isolates from sheep or goats, H- or L- atypical BSE from cattle, scrapie from rodents and sporadic CJD from humans. By contrast, PoPrP-Tg001 mice were susceptible to isolates representative of classical BSE strain regardless the species of origin (cattle, sheep, goat, mouse or human) generating a unique specific PrP<sup>res</sup> profile.

It is known that only a subset of the diversity of possible PrP<sup>Sc</sup> types is compatible with a given PrP primary sequence. In the case of pig-PrP amino acid sequence, our results demonstrate that pig-PrP<sup>C</sup> can only be converted into pig-PrP<sup>Sc</sup> after inoculation with classical-BSE irrespective of the donor species. No infection was achieved with any other PrP<sup>Sc</sup> from the collection of prion strains used in this work. Therefore, conformational flexibility limitations associated to pig-PrP would limit the number of permissible PrP<sup>Sc</sup> conformations compatible between pig-PrP<sup>C</sup> and donor PrP<sup>Sc</sup>. Thus suggesting that pig-PrP<sup>C</sup> amino acid sequence may constitute a paradigm of low conformational flexibility and high resistance to the diversity of prion strains. Amino acid changes of the pig-PrP<sup>C</sup> would be responsible for its limited conformational flexibility, as compared to other more susceptible species. This strengthens the transmission barrier for prion strains others than classical-BSE. This strain may represent a thermodynamically highly favored PrP<sup>Sc</sup> conformation that is readily imprinted on PrP from a range of different species, accounting for the high promiscuity of BSE strain in mammals.

**See also poster P107**

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## **WA10 Prion Disease in Dromedary Camels**

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See O5

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## **WA11 Absence of Classical and Atypical (H- and L-Type) BSE Prions in the Blood of Clinical Bovine Cases as Confirmed by Cattle to Cattle Blood Transfusions**

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While the presence of BSE infectivity in the blood of clinically affected sheep has been demonstrated by blood transfusion experiments in sheep, this question has remained open in the case of BSE-affected cattle. Previous studies on bovine blood lacked either the necessary sensitivity to detect traces of BSE prions and/or utilized only sub-optimal (e.g. wild-type mouse) bioassay systems.

We therefore performed a blood transfusion experiment where between 0.5 and 1 liter of blood of clinical end stage bovines after experimental challenge with either classical or atypical (H- and L-type) BSE was transfused to 4-6 months old calves. For each of the BSE forms, four donor cattle were used for transfusion into eight recipients each, plus two control calves that received a blood transfusion of a BSE negative cow. The animals were kept together in a group in a free-ranging facility for 10 years. From each animal, blood was collected every 6 months, and cerebrospinal fluid (CSF) was collected every 12 months. Starting from 36 months post transfusion, a thorough clinical examination was performed every six weeks in order to detect early symptoms of a BSE infection.

Throughout the experiment, the clinical picture of all animals gave no indication of a BSE infection. Upon necropsy, the brainstem samples were analyzed by BSE rapid tests as well as by the highly sensitive protein misfolding cyclic amplification (PMCA) assay, all giving negative results.

These results prove the absence of BSE infectivity in the blood of BSE affected cattle even when they are in the final clinical phase of the disease. The obtained data are reassuring in terms of the risk assessment for bovine blood and blood products and conclude a three decade long discussion on their BSE status.

See also poster P104

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## **WA12 A scrapie cohort study shows the success of breeding for resistance**

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Introduction. Based on the accumulating evidence of the role of the genetic susceptibility in the occurrence of classical scrapie (CS), breeding for resistance was suggested in the 1990s as a promising strategy to control the disease and was extensively applied. However, population-based studies showing its effectiveness are still missing. Focusing on a large scrapie outbreak occurred in Sardinia (Italy), the aim of this work was to quantify the effectiveness of breeding for resistance to CS by means of a retrospective cohort study based on long-term surveillance data from a large dairy sheep population. Materials and Methods. Within the Sardinian sheep population, three cohorts of flocks have been identified based on a subsequent temporal involvement: cohort 1 (C1), i.e. high genetic merit flocks recruited to breeding for resistance since 2000; C2, i.e. commercial flocks (CF) enrolled on a voluntary basis by the Regional breeding plan (RBP) since 2005; C3, i.e. CF enrolled since 2009 when the RBP was extended to all the flocks. Cohort membership was restricted to the flocks subject to CS active surveillance between 2006 and 2016. On average, annually we considered 154 flocks from C1 (SD 56), 130 from C2 (SD 47) and 1,733 from C3 (SD 610). Poisson regression models were fitted to either flock-level incidence or animal-level prevalence data. Confounding from between-flock heterogeneous surveillance has been controlled through modelling. By entering year as a continuous explanatory variable, adjusted incidence rate ratios (IRR), prevalence ratios (PR) and their confidence intervals (95%CI) were used to identify statistically significant trends. Results. Over the study period, in total 67,523 sheep have been tested from the three cohorts leading to the detection of 194 CS index cases from 179 flocks. Modelling of adjusted incidence and prevalence data showed downward, although not statistically significant trends for C1 (IRR=0.81, PR=0.86) and flat trends for C2 (IRR=0.99, PR=1.00). The decreasing trend in C1 incidence was statistically different from the increasing one of C3 (IRR of the interaction term=0.78, 95%CI 0.62-0.98). Both incidence and prevalence of C3 showed a steady increase up to 2011 and a sharp decrease afterwards (C3 modelling between 2011 and 2016: IRR=0.68, 95%CI 0.57-0.80; PR=0.69, 95%CI 0.59-0.81). Conclusion. For the first time we provide results from a large scale, long term comparison of three sheep cohorts where different periods of breeding for resistance to CS has been applied. The fit for purpose of the breeding for resistance strategy was confirmed through a population-based cohort study that allowed the quantification of a relevant effect on both the incidence and the prevalence of the disease while accounting for the potential confounding effect of surveillance.

**See also poster P106**

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## **WA13 Detection of seeding activity in preclinical blood samples from BSE-infected sheep using protein misfolding cyclic amplification (PMCA)**

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Transmission of variant Creutzfeldt-Jakob disease (vCJD) is a major public health concern, as individuals infected with prions can remain healthy for years before developing signs/symptoms of brain disease. Development of a highly sensitive, non-invasive and inexpensive prion detection assay at preclinical stage is an important goal to minimize further spreading of vCJD. We have used sheep experimentally infected with BSE as a model to study the risks of transmission of prion diseases by transfusion of blood components that are commonly used in humans and collected a large archive of blood samples from animals at different stages of infection. Our aim is to determine the sensitivity of detection by PMCA of seeding activity in preclinical blood samples, and understand the relationship between the level of seeding activity and transmission of infection by blood transfusion.

#### Methods

We used a 96-well microplate-based, miniaturized bead serial PMCA protocol<sup>1-2</sup> with brain homogenate from tgShpXI transgenic mice expressing sheep ARQ PrP as substrate. Reactions were seeded with tenfold serial dilutions of BSE-infected sheep brain homogenate (positive control), buffy coat samples from BSE-infected sheep undiluted or diluted 1:1 in PMCA buffer, and corresponding blood samples from uninfected sheep (negative controls).

#### Results

We were able to detect PrP<sup>Sc</sup> in reactions seeded with a 10<sup>-8</sup> dilution of BSE-infected sheep brain homogenate after 4-6 rounds of serial PMCA, and in blood samples from sheep showing clinical signs of BSE. In the transfusion experiments, blood was collected from sheep orally infected with BSE at 10 months post-infection, at least 8 months before they developed clinical signs, and used to prepare components (e.g. red cells, plasma) for transfusion to recipient sheep. Positive amplification was identified in PMCA reactions seeded with buffy coat samples collected at this time point, with a higher proportion of positive results in samples from donor sheep that transmitted infection to one or more recipients. PMCA testing of buffy coat samples collected from individual sheep at different stages throughout infection have demonstrated positive amplification from 6 months post infection onwards in some animals. No non-specific amplification was detected in negative control samples after up to 8 rounds of serial PMCA.

#### Conclusions

We have confirmed that the microplate-based serial PMCA method demonstrates relatively sensitive and specific detection of infection in preclinical blood samples collected from BSE-infected sheep from at least 6 months post-infection. Our preliminary data suggests that levels of seeding activity are higher in blood samples from sheep that transmitted infection by transfusion, potentially indicating a positive correlation between the amount of misfolded PrP and infectivity titres in blood.

<sup>1</sup>Moudjou *et al.* (2013) MBio.

<sup>2</sup>Lacroux *et al.* (2014) PLoS Pathog.

**See also poster P95**

## WA14 Prion strain evolution and adaptation

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Prions are abnormal conformers (PrP<sup>Sc</sup>) of the host-encoded cellular prion protein (PrP<sup>C</sup>). They replicate by templating the conversion and polymerization of PrP<sup>C</sup>. Multiple strains of prions are recognized phenotypically within the same host species. Prion strains exhibit specific incubation periods, stereotyped clinical signs and neuropathology, and specific tropism for the cerebral and lymphoid tissues. Strains are conformational variants of PrP<sup>Sc</sup> at the level of the tertiary and/or quaternary structure. This usually results in one dominant PrP<sup>Sc</sup> conformation as detected by conventional immunodetection methods, although there is evidence that prions can be composed of several substrains. Substrain selection is expected to occur during adaptation of a prion to a new host: differences in recipient PrP primary sequence are supposed to drive modifications of the conformational rearrangements of the native PrP in order to fit the inoculum. The efficacy of this process differs between the brain and lymphoid tissue, sometimes resulting in a lowered species barrier in the lymphoid tissue. By using transgenic animals expressing PrP at variable levels, we also showed that substrain selection occurred during homotypic transmission events and is controlled by variations in the expression level of host PrP in the brain. The question then arises of similar substrain selection within different tissue of the same infected host due to variations in PrP expression levels, e.g. central nervous system vs. other peripheral organs such as the lymph nodes or the spleen. We will here present data supporting the view that PrP<sup>C</sup> expression levels are instrumental in prion substrain selection and specific tropism for the lymphoid tissue. These results highlight the importance of screening prions in other organs than the central nervous system to apprehend prion diversity and potential of evolution.

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## WA15 The agent of chronic wasting disease from pigs is infectious in transgenic mice expressing human *PRNP*

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We have previously shown that the chronic wasting disease (CWD) agent from white-tailed deer can be transmitted to domestic pigs via intracranial or oral inoculation although with low attack rates and restricted PrP<sup>Sc</sup> accumulation. The objective of this study was to assess the potential for cross-species transmission of pig-passaged CWD using bioassay in transgenic mice.

Transgenic mice expressing human (Tg40), bovine (TgBovXV) or porcine (Tg002) *PRNP* were inoculated intracranially with 1% brain homogenate from a pig that had been intracranially inoculated with a pool of CWD from white-tailed deer. This pig developed neurological clinical signs, was euthanized at 64 months post-inoculation, and PrP<sup>Sc</sup> was detected in the brain. Mice were monitored daily for clinical signs of disease until the end of the study. Mice were considered positive if PrP<sup>Sc</sup> was detected in the brain using an enzyme immunoassay (EIA).

In transgenic mice expressing porcine prion protein the average incubation period was 167 days post-inoculation (dpi) and 3/27 mice were EIA positive (attack rate = 11%). All 3 mice were found dead and clinical signs were not noted prior to death. One transgenic mouse expressing bovine prion protein was euthanized due to excessive scratching at 617 dpi and 2 mice culled at the end of the study at 700 dpi were EIA positive resulting in an overall attack rate of 3/16 (19%). None of the transgenic mice expressing human prion protein that died or were euthanized up to 769 dpi were EIA positive and at study end point at 800 dpi 2 mice had positive EIA results (overall attack rate = 2/20 = 10%). The EIA optical density (OD) readings for all positive mice were at the lower end of the reference range (positive mice range, OD = 0.266-0.438; test positive reference range, OD = 0.250-4.000).

To the authors' knowledge, cervid-derived CWD isolates have not been successfully transmitted to transgenic mice expressing human prion protein. The successful transmission of pig-passaged CWD to Tg40 mice reported here suggests that passage of the CWD agent through pigs results in a change the transmission characteristics which reduces the transmission barrier of Tg40 mice to the CWD agent. If this biological behavior is recapitulated in the original host species, passage of the CWD agent through pigs could potentially lead to increased pathogenicity of the CWD agent in humans.

**See also poster P93**

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## **WA16 Monitoring Potential CWD Transmission to Humans**

Belay ED

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The spread of chronic wasting disease (CWD) in animals has raised concerns about increasing human exposure to the CWD agent via hunting and venison consumption, potentially facilitating CWD transmission to humans. Several studies have explored this possibility, including limited epidemiologic studies, in vitro experiments, and laboratory studies using various types of animal models. Most human exposures to the CWD agent in the United States would be expected to occur in association with deer and elk hunting in CWD-endemic areas. The Centers for Disease Control and Prevention (CDC) collaborated with state health departments in Colorado, Wisconsin, and Wyoming to identify persons at risk of CWD exposure and to monitor their vital status over time. Databases were established of persons who hunted in Colorado and Wyoming and those who reported consumption of venison from deer that later tested positive in Wisconsin. Information from the databases is periodically cross-checked with mortality data to determine the vital status and causes of death for deceased persons. Long-term follow-up of these hunters is needed to assess their risk of development of a prion disease linked to CWD exposure.

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## **WA17 Vaccination of transgenic elk PrP mice and reindeer with recombinant prion protein overcomes self-tolerance and protects mice against CWD infection**

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Chronic wasting disease (CWD) is a fatal and highly infectious neurodegenerative disease that affects various cervid populations in North America and now Europe. No effective measures are currently available to control CWD. Vaccination would be an important approach to contain the disease. We hypothesized that active vaccination with homologous and aggregation-prone recombinant prion protein (PrP) could induce auto-antibody production against the cellular isoform of PrP (PrP<sup>C</sup>) which would interfere in prion neuroinvasion and be protective against CWD infection from peripheral routes. Five groups (10 mice each) of transgenic mice expressing elk PrP (TgElk) were vaccinated, with 5 doses of either the adjuvant CpG alone, or one of four recombinant PrP immunogens: deer dimer (Ddi), deer monomer (Dmo), mouse dimer (Mdi), and mouse monomer (Mmo). Mice were then challenged intraperitoneally with elk CWD prions and monitored for development of clinical signs. The group receiving Mmo had the highest antibody titers in ELISA, and in immunoblot post-immune sera from this group showed the best detection of PrP from elk CWD samples. Induced antibodies bound to authentic PrP<sup>C</sup> and PrP<sup>Sc</sup>, blocked in RT-QuIC and reduced PrP<sup>Sc</sup> in cultured cells. Importantly, all 4 vaccinated groups survived longer than the CpG control group. Interestingly, the Mmo-immunized group revealed 70% prolongation of the median survival time compared to the control group (185 vs. 109 dpi). Dmo and Ddi groups had prolongations of 57% and 30%, respectively (172 and 142 dpi). We tested for prion infection in brain and spleen of all clinically sick mice. Notably, the attack rate was 100% as shown by positive signals of CWD in all tested tissues using 3 different readouts (WB, RT-QuIC and IHC). Our pilot study in reindeers showed appreciable humoral immune responses to Mdi and Ddi immunogens, and the post-immune sera from the Ddi vaccinated reindeer mitigated CWD propagation in a cell culture model (CWD-RK13). Taken together, our study provides highly promising vaccine candidates against CWD, however, further studies in cervids are required to investigate vaccine efficacy in the natural CWD hosts.

**See also poster P100**

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# Posters

## **I. Structure and basic biology of PrP<sup>Sc</sup> and other mammalian, yeast and fungal prions**

### **P1 Structure of prion $\beta$ -oligomers as determined by short-distance crosslinking constraint-guided discrete molecular dynamics simulations.**

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The conversion of the native monomeric cellular prion protein (PrP<sup>C</sup>) into an aggregated pathological  $\beta$ -oligomeric (PrP <sup>$\beta$</sup> ) and infectious form (PrP<sup>Sc</sup>) is the central element in the development of prion diseases. The structure of the aggregates and the molecular mechanisms of the conformational change involved in the conversion are still unknown. We have applied a combination of chemical crosslinking, hydrogen/deuterium exchange (HDX), limited proteolysis, and surface modification with mass spectrometry for the differential characterization of the native and the urea-acid converted prion  $\beta$ -oligomer structures to get an insight into the mechanism of the conversion and aggregation. For the determination of the structure of the monomer and dimer unit of the  $\beta$ -oligomer we have applied a recently-developed approach for de novo protein structure determination based on the incorporation of zero-length and short-distance crosslinking data as constraints in discrete molecular dynamics simulations (CL-DMD). To distinguish intra-



protein from inter-protein constraints we have applied crosslinking to the equimolar mixture of  $^{14}\text{N}/^{15}\text{N}$ -metabolically labelled  $\beta$ -oligomer. Using HDX, we have detected a region of the protein in which backbone amides become more protected from exchange due to formation of new  $\beta$ -structure in the PrP $\beta$  compared to PrP $\alpha$ . By comparing the structures before and after the conversion, we deduce the conformational change, that occurs during the conversion as the rearrangement and disassembly of the beta sheet 1 – helix 1 – beta sheet 2 ( $\beta$ 1-H1- $\beta$ 2) region from the helix 2 – helix 3 (H2-H3) core, resulting in stretching and the exposure of hydrophobic segments of the protein chain leading to the formation of the new  $\beta$ -sheet nucleation site and inter-protein contacts within the aggregates. We were able to assemble and validate a structure of the  $\beta$ -oligomer using all the structural proteomics experimental data. The proposed  $\beta$ -oligomer assembly gives a clue to how template-based conversion, growth of the aggregates and maturation into fibrils may occur.

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## **P2 Comparison of linear characteristics of Sup35NMp fibrils on Au, Si, formvar and mica surfaces using AFM, SEM and TEM**

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Prion and some other incurable human neurodegenerative diseases are associated with misfolding of specific proteins, followed by the formation of amyloids. Despite the widespread usage of the transmission electron (TEM) and the atomic force microscopy (AFM) for studying such aggregates, many related methodological issues still have not been studied. Here, we consider one of the first amyloids found in *Saccharomyces cerevisiae* yeast, i.e. Sup35NMp, to study the adsorption of monomeric protein and its fibrils on the surface of mica, silica, gold and on formvar film. Comparison of linear characteristics of these units calculated by processing of images obtained by AFM, TEM and scanning electron microscopy (SEM) was carried out. We analyzed for the first time the process of adsorption of monomeric Sup35NMp on different surfaces and found that this protein forms a film on mica while no films are formed on silicon, gold, and formvar. Upon excess of the monomeric protein the film may comprise several layers with thickness of each layer being approximately equal to 0.7 nm. Analysis of the fibrils' immobilization on different substrates testified that the formation of the film reduces the efficiency of fibrils fixation. We believe that it is optimum to incubate substrates in a solution containing a protein at a concentration of  $\sim 30 \mu\text{g/mL}$ , not longer than 100 s. Under these conditions, the fibrils are fixed separately and the adsorbed monomeric protein has no significant effect on their linear dimensions. We demonstrated that the fibrils immobilization rate is varied for different substrates. The rate is similar for gold and mica while it is higher for silicon. The spread of the fibrils' diameters is also varied for different surfaces. The smallest variation of diameter is observed during measurements on mica using AFM, on silicon using SEM and on formvar film using TEM. Under these conditions, it is necessary to measure the mean diameter of at least 50–70 fibrils to obtain the values in the confidence interval (95%) of  $\pm 1$  nm. We have also shown that TEM underestimates and SEM overestimates the value of fibrils diameter as

compared to that of AFM. Other linear characteristics of fibril do not vary significantly for different surfaces. Accurate measurements of the fibril cross section allowed to suppose that not the whole polypeptide chain forms the fibrils core. The morphology of the fibril edges is consistent with the assumption that M domain is outside the fibril core and may form the “beta-sheet in register” structure with the same domains of adjacent protein molecules.

The research was supported by research resource centers of St Petersburg State University, funded by the grant of the President of the Russian Federation (MK-512.2017.4) and by the Russian Foundation for Basic Research (17-54-150002).

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### **P3 Properties of Prion Self-replication**

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### **P4 Prion:lipid analysis using membrane nanodiscs bounded by amphipathic polymers**

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### **P4B A new solid-state NMR approach to determine 3D structures of prion amyloid fibrils at atomic resolution**

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### **P5 Recombinant Antibody Fragments as Tools for Structural Analyses of BSE and other Prions**

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Prion diseases are rare, inexorably progressive, and fatal neurodegenerative disorders that affect individuals in the prime of their life, with no therapy other than palliation. They are unique compared to other neurodegenerative disorders in that they are transmissible, whereby the infectious state (PrP<sup>Sc</sup>) is propagated through the reproduction of its secondary, tertiary, and quaternary structure. Prion diseases are associated with the deposition of PrP<sup>Sc</sup>, a misfolded variant of the cellular prion protein, PrP<sup>C</sup>, which is a normal cell surface glycoprotein and is widely expressed in the nervous system. Bovine Spongiform Encephalopathy (BSE) is an acquired prion disease in cattle, colloquially named “mad cow” disease. It was first described in 1986 in the UK and has raised serious concerns through its transmission to humans, where it is known as variant Creutzfeldt-Jakob disease (vCJD).

The structure of recombinant PrP is extensively studied using various biophysical techniques, which have revealed a C-terminal globular domain and an N-terminal flexible tail. So far, no high-resolution structure of PrP<sup>Sc</sup> has been published, and only its overall architecture as a four-rung  $\beta$ -solenoid is known. Fab fragments (Fabs) are small antibody derivatives that maintain antigen-binding capacity, and can be used to study the structure of their antigens. One of the advantages of Fabs over intact antibodies is their small size, which allows them to penetrate deeper into protein aggregates, such as amyloid fibrils. In order to obtain further insights into the structure of PrP<sup>Sc</sup>, we are using recombinant Fabs as tools to determine the accessibility of specific epitopes. This is done under native and denatured conditions using immunogold labelling, electron microscopy, and difference mapping. A Fab library was generated through an *in vitro* phage display system targeting four main regions within PrP: charged cluster 1, octapeptide repeats, charged cluster 2, and globular domain.

Individual Fabs were expressed in *E. coli*, purified, and tested for their ability to bind PrP in dot blot and western blot assays, which served as quality control measures. Epitope mapping indicated that individual Fabs recognized linear or discontinuous epitopes, and some were able to bind native PrP<sup>Sc</sup> fibrils. Complexes between Fabs and purified BSE prion fibrils are being analyzed using immunogold labelling and electron microscopy. Electron micrographs of suitable prion fibrils will be used to generate 3D reconstructions. Differences between labeled and unlabeled fibrils will provide insights into the orientation of the respective epitopes and describe which residues participate in the  $\beta$ -solenoid core of PrP<sup>Sc</sup>. We also plan on analyzing complexes between Fabs and recombinant PrP (recPrP) using X-ray crystallography to obtain atomic resolution structures. Together these analyses will provide insights into the conformational changes that occur during the conversion from PrP<sup>C</sup> to PrP<sup>Sc</sup>.

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## **P6 Mass-Per-Length Analysis of Infectious Human Prions**

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Human prion diseases present as sporadic, familial, infectious, or iatrogenic forms, and include diseases such as Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), and Fatal Familial Insomnia (FFI). The cause of the wide range of phenotypic variation in the human prion diseases is unknown, but the pathology is caused by aberrantly folded versions of the prion protein, termed PrP<sup>Sc</sup>. Evidence indicates that distinct aggregated forms of PrP<sup>Sc</sup> with heterogeneous morphologies regarding fibril length, width, shape, longitudinal twist, and the number of protofilaments may characterize the distinct disease entities. The insolubility of PrP<sup>Sc</sup> and its overall propensity to aggregate make it inaccessible to high-resolution structure analysis techniques. Nevertheless, low-resolution approaches have provided enough information on the architecture of PrP<sup>Sc</sup> and to devise molecular models that contribute to elucidate its structure. To date, the main, competing proposals include a parallel in-register intermolecular  $\beta$ -sheet structure (PIRIBS) and a four-rung  $\beta$ -solenoid fold. In the PIRIBS model, each molecule of PrP<sup>Sc</sup> contributes only 4.8 Å of height to the prion fibril, while in the four-rung  $\beta$ -solenoid model each molecule of PrP<sup>Sc</sup> contributes 19.2 Å (4 x 4.8 Å). In the PIRIBS model, it is required that two “protofilaments” are connected, in order to accommodate the mass of the entire PrP<sup>Sc</sup> molecule (i.e. limiting prion fibrils to an even number of “protofilaments”), while in the  $\beta$ -solenoid model each protofilament constitutes a separate assembly of stacked PrP<sup>Sc</sup> molecules.

Using tilted-beam transmission electron microscopy (TB-TEM), we are measuring the mass-per-length (MPL) value of individual PrP<sup>Sc</sup> fibrils derived from human CJD and GSS brain samples. By determining the number of protofilaments per fibril, we will assess the heterogeneity of individual prion samples. These results will provide a platform to evaluate the proposed models for the structure of PrP<sup>Sc</sup> fibrils. Tobacco Mosaic Virus (TMV), with its well-determined structure and MPL value of 131 kDa / nm, serves as an internal calibration standard for the MPL measurements. Preliminary results obtained with purified PrP<sup>Sc</sup> fibrils from sporadic CJD samples indicated a population of fibrils with an MPL value around 60 kDa / nm. Additional MPL measurements with GSS patient-derived samples are underway. Recombinant PrP amyloid fibrils as well as *in vitro* converted recombinant PrP<sup>Sc</sup> will be used to round out the MPL comparison of prion fibril structures.

MPL data impose a stringent constraint on viable fibril models when the subunit mass is known. By calibrating the image intensity of PrP<sup>Sc</sup> to the internal standard, we will be able to limit the number of possible indexing schemes significantly, and we will provide independent experimental validation to the current proposed models.

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#### **P7 Differences in the folding dynamics of prion proteins from species with different disease susceptibility observed at the single-molecule level**

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#### **P8 Detection of amyloid fibrils using plasmonic chirality**

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The detection of amyloid fibrils at low concentration is pivotal in the early diagnosis of several neurodegenerative disorders and development of therapeutic strategies. Herein, we report a novel methodology for the specific identification of amyloid fibrils using chiroptical effects in plasmonic nanoparticles. The formation of amyloid fibrils based on  $\alpha$ -synuclein was probed using gold nanorods, which showed no apparent interaction with monomeric proteins but effective adsorption onto fibril structures via noncovalent interactions. The amyloid structure drives a helical nanorod arrangement, resulting in intense optical activity at the surface plasmon resonance wavelengths. This sensing technique was successfully applied to brain homogenates of patients affected by Parkinson's disease, wherein disease-related fibrils were detected through chiral signals from gold nanorods in the visible and near IR, whereas healthy brain samples did not exhibit any meaningful optical activity. The technique was extended to the specific detection of infectious recombinant prions, confirming the wide potential of the technique. The intense chiral response driven by strong dipolar coupling in helical gold nanorod arrangements allowed us to detect amyloid fibrils down to nanomolar concentrations.

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## **P9 Interaction between human Prion Protein and Amyloid beta oligomers as seen by solid-state MAS-NMR**

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Both prion protein (PrP) and Amyloid beta (A $\beta$ ) play an important role in the pathogenesis of neurodegenerative disorders if they misfold or form amyloid fibrils. A $\beta$  oligomers have been identified as neurotoxic factor of the pathogenesis of Alzheimer's disease, whereas fibrils are known to be the main component of insoluble plaques [1]. While 3D structures of different A $\beta$ (1-42) fibril types have recently been determined at high resolution [2-4], secondary structure elements of the oligomers are relatively unknown, due to their transient nature [5]. Recently, it has been shown that oligomeric A $\beta$ (1-42) binds human PrP (huPrP) with high affinity [6-8].

We investigated complexes formed by A $\beta$  oligomers and huPrP by solid-state NMR-spectroscopy. Experiments were performed on different samples where either huPrP or A $\beta$  was uniformly  $^{13}\text{C}/^{15}\text{N}$  isotope labelled and therefore visible in the NMR-spectra. For full-length (aa 23 to 230) as well as N-terminal huPrP (aa 23 to 144), the N-terminal part was found to be immobilized but lacking a defined secondary structure, whereas the C-terminal part did not seem to be involved in oligomer binding.

For A $\beta$  oligomers, secondary chemical shifts point towards a substantial  $\beta$ -sheet content. Conformational heterogeneity was observed for restricted regions in the amino acid sequence. Nevertheless, a partial resonance assignment was possible.

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## P10 Structure of well-ordered A $\beta$ (1-42) amyloid fibrils

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Amyloids are implicated in neurodegenerative diseases. Fibrillar aggregates of the amyloid- $\beta$  protein (A $\beta$ ) are the main component of the senile plaques found in brains of Alzheimer's disease patients. We investigated well-ordered A $\beta$ (1-42) fibrils by cryo-electron microscopy (cryo-EM) as well as solid-state NMR spectroscopy [1]. Solid-state NMR spectra are indicative of a high degree of homogeneity on the molecular level, thus confirming that the fibril structure determined by cryo-EM to 4.0 Å resolution is representative for the complete sample. Fibrils were found to be composed of two intertwined protofilaments. The backbone of all 42 residues and nearly all sidechains are well resolved in the EM density map and also visible in dipolar based solid-state NMR-spectra; in contrast to most Ab fibril structures also the entire N-terminus is part of the cross- $\beta$  structure resulting in an overall "LS"-shaped topology of individual subunits. The dimer interface protects the hydrophobic C-termini from the solvent. The unique staggering of the non-

planar subunits results in markedly different fibril ends, termed "groove" and "ridge", leading to different binding pathways on both fibril ends, which has implications for fibril growth.

Low-temperature solid-state NMR spectra and NMR relaxation data give further information about flexibility and dynamics of the protein chain.

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### **P11 Full-length human prion protein displays a whole variety of conformational transitions as observed by high hydrostatic pressure (HHP), xenon as a probe and spin labelling of the N-terminal domain**

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### **P12 A bacterial prion-like protein as a synthetic model to study amyloid toxicity**

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### **P13 Prion strain interference in the absence of conversion**

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Prion strains are encoded by strain-specific conformations of PrP<sup>Sc</sup> that can differ in relative susceptibility to inactivation, pathogenicity, and host range. In both animal and human prion diseases, multiple strains can coexist in an individual host. When present together, prion strains can interfere with the replication of the other strain affecting strain emergence. For example, inoculation of the drowsy (DY) strain of transmissible mink encephalopathy (TME), the blocking

strain, prior to superinfection with the hyper (HY) strain of TME can result in DY TME extending the incubation period of HY TME or completely blocking HY TME from causing disease. In these studies, and others, replication of the blocking strain corresponded with its ability to interfere with the superinfecting strain. It is unknown, however, if prion conversion per se is required or if the presence of PrP<sup>Sc</sup> is sufficient for strain interference to occur.

While investigating the environmental fate of prions we found conditions that disassociated prion conversion activity from the presence of PrP<sup>Sc</sup>. Specifically, 10 repeated cycles of wetting and drying of DY TME bound to silty clay loam (SCL) did not result in a significant reduction of PK resistant PrP<sup>Sc</sup>, however, this material failed to serve as a template for conversion in protein misfolding cyclic amplification (PMCA). We tested the hypothesis that prion conversion is not required for strain interference to occur using this PMCA replication deficient DY PrP<sup>Sc</sup> in PMCA strain interference (PMCA<sub>Si</sub>). In the absence of wetting and drying treatment, DY and HY TME bound to SCL were able to amplify in PMCA. When this material was subjected to PMCA<sub>Si</sub>, HY emergence was delayed indicating that SCL bound DY TME that can convert in PCMA can interfere with the emergence of HY PrP<sup>Sc</sup> consistent with previous results. Using SCL bound replication deficient DY PrP<sup>Sc</sup>, HY PrP<sup>Sc</sup> emergence was delayed compared to samples seeded with HY PrP<sup>Sc</sup> alone. PMCA conversion deficient DY PrP<sup>Sc</sup> can interfere with and delay the emergence of HY PrP<sup>Sc</sup> in PMCA<sub>Si</sub>. This data suggests that prion conversion is not required for prion interference to occur.

Several mechanisms exist to explain strain interference in the absence of blocking strain conversion. Efficient conversion of untreated, soil-bound DY PrP<sup>Sc</sup> indicates that the nascent binding site of PrP<sup>Sc</sup> remains accessible to bind PrP<sup>C</sup> after SCL binding and retains conformational flexibility to support prion conversion. Drying DY PrP<sup>Sc</sup> to SCL may result in overstabilization of PrP<sup>Sc</sup> to the soil surface preventing prion conversion yet leaving the nascent binding site of PrP<sup>Sc</sup> available to bind PrP<sup>C</sup> sequestering it from HY PrP<sup>Sc</sup> resulting in strain interference.

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#### **P14    Divalent cation effects on elk prion protein stability and fibril formation**

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Misfolding of the normally folded prion protein of mammals (PrP<sup>C</sup>) into infectious fibrils causes a variety of diseases, including scrapie in sheep, chronic wasting disease (CWD) in cervids, and bovine spongiform encephalopathy (BSE) in cattle. The misfolded form of PrP<sup>C</sup>, termed PrP<sup>Sc</sup>, or in the case of CWD, PrP<sup>CWD</sup>, interacts with PrP<sup>C</sup> to create more PrP<sup>CWD</sup>. This process is not clearly defined but is affected by the presence and interactions of biotic and abiotic cofactors. These include nucleic acids, lipids, glycosylation, pH, and ionic character. PrP<sup>C</sup> has been shown to act as a copper-binding protein *in vivo*, though it binds to other divalent cations as well. The significance of this binding has not been conclusively elucidated. Previous reports have shown that metal binding sites occur throughout the N-terminal region of PrP<sup>C</sup>. Other cations like manganese have also been shown to affect PrP<sup>C</sup> abundance in a transcript-independent fashion. Here, we examined the ability of different divalent cations to influence the protein stability and *in vitro* conversion efficiency of two variants of PrP from elk (M132 and L132) in the mature form of the prion protein (amino acids 26-234). We find that copper and zinc both de-stabilize PrP. We also find that PrP M132 exhibits a greater degree of divalent cation induced destabilization than L132. This supports findings that leucine at position 132 confers resistance to CWD, while M132 is more susceptible. However, *in vitro* conversion of PrP is suppressed equally by either copper



or zinc, in both L132 and M132 PrP. This report demonstrates the complex importance of ionic character on the PrP<sup>C</sup> folding pathway selection on the route to PrP<sup>Sc</sup> formation.

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#### **P15 Heparan sulfate modulates the brain distribution of fibrillar prions**

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#### **P15B PrP glycans modify prion aggregate conformation in a strain-dependent manner**

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Unlike other amyloidogenic proteins, prions rarely form fibrillar, congophilic plaques in the brain. PrP has two variably-occupied, N-linked glycan attachment sites, and a missense mutation that prevents one glycan attachment causes familial prion disease with unusual clinical features and pathology (1). Mutating the glycan attachment sites also impacts the disease phenotype and prion strain properties in mouse models (2). Yet how the glycans impact prion assembly into fibrils and spread through the CNS remains unclear. To investigate how PrP glycans modify the prion conformation, four mouse-adapted prion strains were inoculated intracerebrally into newly developed knock-in mice that lack glycans on PrP. Thus far, we found remarkable differences among the strains, in that two prion strains showed no major differences in the disease phenotype or PrP<sup>Sc</sup> biochemical properties, whereas one strain showed profound differences in PrP<sup>Sc</sup> conformation, forming abundant large extracellular plaques as compared to diffuse deposits and small plaques in infected wild type mice. Studies are ongoing to test whether the phenotypic differences depend on the cellular conversion site. Grasbon-Frodl E, Lorenz H, Mann U, Nitsch RM, Windl O, Kretzschmar HA. Loss of glycosylation associated with the T183A mutation in human prion disease. *Acta Neuropathol.* 2004;108(6):476-84. Cancellotti E, Bradford BM, Tuzi NL, Hickey RD, Brown D, Brown KL, Barron RM, Kisielewski D, Piccardo P, Manson JC. Glycosylation of PrP<sup>C</sup> determines timing of neuroinvasion and targeting in the brain following transmissible spongiform encephalopathy infection by a peripheral route. *J Virol.* 2010;84(7):3464-75.

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#### **P16 Regulation of Amyloid Aggregation by Adjacent Sequences**

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Cross-beta fibrous aggregates (amyloids) and their transmissible forms (prions) cause diseases in mammals and control heritable traits in yeast. Amyloids and prions are widespread in biological systems and may play positive biological roles, e. g. in microbial adaptation, melanin polymerization, and memory. About 1% a human proteome is composed of proteins with domains that are similar by amino acid composition to prion domains of yeast proteins. *In vitro*, almost all proteins can form amyloids under certain conditions. However, it is still not understood how toxic amyloid conformation is suppressed for majority of the proteins in physiological conditions, and what enables some proteins to overcome this suppression.

Some eukaryotic proteins with amyloidogenic regions contain additional domains that prevent the transition to toxic amyloid aggregates, and/or promote the formation of other assemblies such as liquid droplets and hydrogels. Despite the important role of domains adjacent to amyloidogenic regions in regulation of amyloidogenic their properties, mechanisms of such regulation are not well understood.

The purpose of our work is to elucidate the mechanism of the regulation of amyloid formation by regions adjacent to amyloidogenic domains, employing *in vivo* aggregation in yeast cells as a model.

Our data show that adjacent domains influence aggregation properties of amyloidogenic regions. The fusion of mammalian Abeta peptide to prion domain of the yeast Sup35 protein (Sup35N-Abeta) promotes its aggregation and allows the chimeric protein to aggregate under conditions where Sup35 does not aggregate. However, the addition of sequences to both sides of the amyloid beta (Sup35N-Abeta-CFP) inhibits formation of amyloid aggregates, leading to the formation of non-amyloid-type assemblies instead. Moreover, attachment of the fluorescent protein (CFP or YFP) to the N-terminus of Abeta drastically reduces aggregate formation compared with the C-terminal attachment. Thus, mode of aggregation of amyloid regions depends on the nature and location of adjacent sequences. We hypothesize that the proximity to large structured or globular domains disrupts and in some cases, (even to potentially amyloidogenic domains not forming amyloids in given conditions) impairs amyloid formation aggregation due to steric hindrance, and in some case, promotes formation of alternative assemblies. This effect is increased when adjacent sequences are located on both sides of the amyloidogenic region.

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## **P17 Differences in the efficiency of prion infection of CAD5 cells by distinct fractions of extracellular vesicles**

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The key event in the pathogenesis of prion diseases is autocatalytic conversion of cellular prion protein (PrP<sup>C</sup>) to its pathological form (PrP<sup>TSE</sup>) resulting in neuropathy and inevitable death of an organism. Direct physical contact of PrP<sup>C</sup> with PrP<sup>TSE</sup> is needed for the conversion which includes change in the secondary and tertiary structure of PrP<sup>C</sup> molecule. Spread of PrP<sup>TSE</sup> in the organism is essential prerequisite for the disease pathogenesis. Three ways of PrP<sup>TSE</sup> spreading among the cells in cell culture models were described – by direct cell to cell contact, by “tunnelling nanotubes” and by extracellular vesicles (EVs). Even though spread by direct cell contact seems to be more efficient, the EVs have the capability of long distance spreading of PrP<sup>TSE</sup> within the organism and due to their small size even through hematoencephalic barrier. Three basic types of EVs are recognized – apoptotic bodies, microvesicles (MVs) emerging by budding from the cellular plasma membrane and exosomes emerging by exocytosis from multivesicular bodies. Our study was focused on MVs and exosomes produced by murine catecholaminergic cell line CAD5 infected with RML prions. EVs were isolated from conditioned medium by differential centrifugation. MVs, with reported size from 100 – 1000 nm, were pelleted by 14 000x g centrifugation and exosomes, with reported size 30 – 100 nm, were pelleted by 110 000x g ultracentrifugation. The isolated fractions were characterized by cryo-electron microscopy and western blot utilizing distinct protein markers (TSG-101, HSP70, PrP,  $\beta$ -1 integrin and calnexin). Isolated fractions were subsequently used to infect naive CAD5 cells. Interestingly, MVs fraction had notably higher infectivity compared to exosome fraction despite its lower protein concentration. To confirm our results, we conducted infectious experiments with conditioned medium gradually depleted of EVs fractions. Again, the prion infectivity associated with MVs fraction was higher than in exosome fraction. Our results suggest that MVs budding from the cytoplasmic membrane may prove to be more important in the spread of PrP<sup>TSE</sup> within the organism than currently extensively studied exosomes.

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## **P18 Synthetic prions selection and adaptation**

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Prion pathologies are characterized by the conformational conversion of the cellular prion protein (PrP<sup>C</sup>) into a pathological infectious isoform, known as PrP<sup>Sc</sup>. The latter acquires different abnormal conformations, which are associated with specific pathological phenotypes. Recent evidence suggests that prions adapt their conformation to changes in the context of replication. This phenomenon is known as either prion selection or adaptation, where distinct conformations of PrP<sup>Sc</sup> with higher propensity to propagate in the new environment prevail over the others. Here we show that a synthetically generated prion isolate, previously subjected to PMCA amplification and then injected in animals, is able to change its biochemical and biophysical properties according to the context of replication. In particular, in second transmission passage *in vivo* two different prion isolates were found: one characterized by a predominance of the monoglycosylated band (PrP<sup>Sc</sup>-M) and the other characterized by a predominance of the diglycosylated one (PrP<sup>Sc</sup>-D). Neuropathological, biochemical and biophysical assays confirmed that these PrP<sup>Sc</sup> possess

distinctive characteristics. Finally, PMCA analysis of PrP<sup>Sc</sup>-M and PrP<sup>Sc</sup>-D generated PrP<sup>Sc</sup> (PrP<sup>Sc</sup>-PMCA) whose biophysical properties were different from those of both inocula, suggesting that PMCA selectively amplified a third PrP<sup>Sc</sup> isolate. Taken together these results indicate that the context of replication plays a pivotal role in either prion selection or adaptation. By exploiting the ability of PMCA to mimic the process of prion replication *in vitro* it might be possible to assess how changes in the replication environment influence the phenomenon of prion selection and adaptation.

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## **P19 Efficient interspecies transmission of synthetic prions**

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Prions (PrP<sup>Sc</sup>) are the misfolded self-propagating conformation of the cellular protein, PrP<sup>C</sup>. Prion strain properties are encrypted in the conformation of PrP<sup>Sc</sup>. Prions can be synthetically generated *in vitro* by incubating bacterially expressed recombinant prion protein with RNA and endogenous lipids. Synthetic prions can cause disease in animals following intraspecies transmission. It is unknown, however, if synthetic prions can cross the species barrier following interspecies transmission.

To investigate this, we inoculated murine synthetic prions into Syrian hamsters. We found that all of the animals developed clinical signs of prion disease and the CNS contained PrP<sup>Sc</sup> and spongiform degeneration, all consistent with prion infection. Serial intraspecies transmission resulted in a shortening and stabilization of the incubation period consistent with prion adaptation. Clinically, these animals are characterized by a progressive weight gain and relatively long duration of the clinical phase of disease compared to other hamster-adapted prion strains. Western blot migration of first through third hamster passage maintained a 21 kDa migration pattern of the unglycosylated PrP<sup>Sc</sup> polypeptide similar to most hamster-adapted prion strains. The protein misfolding cyclic amplification conversion coefficient from these animals indicated a relatively slow rate of PrP<sup>Sc</sup> formation. Conformational PrP<sup>Sc</sup> stability assays indicated a low conformational stability that remained consistent from first to third passage. Interestingly, the migration, conformational stability, and conversion efficiency of PrP<sup>Sc</sup> from this strain is similar to 139H. Consistent with this observation, the progressive weight gain observed is also a feature of 139H.

Based on these clinical observations and the biochemical properties of PrP<sup>Sc</sup> we hypothesize that the synthetic strain is a rederivation of 139H. This suggests that the synthetic murine fibrils contain the 139A strain. Overall, this is the first description of interspecies transmission of synthetic prions.

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## **P20 The alteration characteristics of [PSI<sup>+</sup>] prion by nonsense mutations in *SUP35* gene**

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The Sup35 (eRF3) protein in complex with Sup45 (eRF1) is required for termination of translation (Zhouravleva et al., 1995; Stansfield et al., 1995). The partial inactivation of Sup35 caused by its aggregation in the  $[PSI^+]$  cells (Patino et al., 1996; Paushkin et al., 1996) leads to the impaired termination of translation, which results in omnipotent nonsense suppression. Different mutations in the *SUP35* gene lead to the same effect. Previously, we have shown that some *sup35* nonsense mutations (*sup35-n*) do not completely abolish accumulation of full size Sup35p (Chabelskaya et al., 2004). This residual amount of full-size Sup35p is probably a reason of occasional readthrough of the nonsense codon due to the reduced amount of the eRF3 (Sup35p) termination factor. Strains with the *sup35-n* mutations exhibit a suppressor phenotype and remain viable in various  $[psi^-]$  genotypic backgrounds. In this work, four mutations located in different parts of *SUP35* gene were studied. It was shown that in haploid yeast cells when combined with  $[PSI^+]$ , these mutations demonstrated synthetic lethality, likewise *sup45-n* mutations (Kiktev et al., 2007). This lethal effect might be explained by a critical depletion of functional Sup35p in the cells bearing two strong nonsense-suppressors. However, it was possible to obtain diploid cells bearing some *sup35-n* mutations together with  $[PSI^+]$ . The presence of these mutations in combination with WT *SUP35* modified the  $[PSI^+]$  characteristics including the aggregate size and level of nonsense suppression. Cells bearing the *sup35-n* mutations together with reduced amount of full-length Sup35p also contain short N-terminal Sup35p fragments that potentially can serve as seeds for  $[PSI^+]$  prion formation. In accordance with this idea, we have shown that some *sup35-n* mutations are able to induce *de novo* appearance of the  $[PSI^+]$  prion. Short *SUP35* fragments expressed in *E. coli* cells were able to aggregate *in vitro* more effectively than the Sup35NM fragment which was estimated by the efficiency of ThT binding. Analysis of the morphology of the aggregates formed by truncated Sup35p fragments by transmission electron microscopy demonstrated their different appearances compared with Sup35NM fibers. The mechanisms providing the phenotypic variability of haploid and diploid yeast strains are discussed.

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## **P21 A New Structural Model of PrP<sup>Sc</sup> Based on Recent Experimental Data and Computational Techniques.**

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## **P22 Searching for disappearing prions: the mechanism behind photodynamic inactivation by phthalocyanines**

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**Aims:** The resistance of prions to conventional sterilization techniques and their high affinity to surfaces of various origin are raising the risk of nosocomial transmission from asymptomatic carriers by contaminated medical tools. Previously, we have demonstrated the efficiency of photodynamic inactivation (PDI) of prions by minimally toxic disulfonated hydroxyaluminium phthalocyanine (PcDS) [1]. Recently, we have discovered different sensitivity of prion strains to PDI (unpublished data). In this study we aimed to elucidate the mechanism behind the PcDS PDI of prions.

**Methods:** 1% (w/v) brain homogenate (BH) of terminally ill mice infected with RML was utilized as a source of prions. The PDI of PrP<sup>TSE</sup>/PrP<sup>C</sup> or recombinant human PrP121-230 (rHuPrP) in the presence of PcDS was performed on high-throughput microplate Diode Array Light Source equipped with 96 diodes ( $\lambda_{em}=655$  nm). The species responsible for PDI were determined by modified iodide detection method [2], quenching reaction (NaN<sub>3</sub>) and degradation of uric acid. Prion protein was detected by western blot with antibodies (Ab) against different parts of the molecule. The whole protein content was monitored by BCA assay and AgNO<sub>3</sub> staining in PAGE gels.

**Results:** Singlet oxygen was the only reactive oxygen species detected during the PDI and its oxidation ability was confirmed by photobleaching of uric acid. PDI led to PrP<sup>TSE</sup>/PrP<sup>C</sup> elimination below the detection limit of WB (0.03% BH) after 90 min of irradiation (0.5 µg/mL PcDS) when Ab against the middle part and C-termini (BE12, 6D11, D18, AH6 and GE8) were used. However, remains of PrP<sup>TSE</sup>/PrP<sup>C</sup> with lower electrophoretic mobility were detected with Ab against N-termini (DC2 and AG4) and were found to decrease with increasing PDI intensity. Interestingly, the total protein content did not fall below 90% of its initial concentration. Protein bands stained in the gel showed similar profile with slightly decreasing intensity correlated with increasing PcDS concentration at PDI, but no signs of increased aggregation. Likewise, rHuPrP also decreased its electrophoretic mobility and was detected by silver staining, but not by Abs after the PDI.

**Conclusions:** Our results suggest that PDI in the presence of PcDS initiates prion protein modification by singlet oxygen rather than its degradation. The modification is accompanied by significant decrease of prion infectivity [1]. It remains to be elucidated whether the PrP remains detected by N-termini antibodies originate from PrP<sup>TSE</sup>, PrP<sup>C</sup> or both as the Ab epitopes are gone after PK treatment.

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[1] Janouskova O, et al. J Gen Virol 2012; 93:2512-17

[2] Mosinger J, et al. Experimentia 1995; 51:106

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## **P23 The transcriptional regulator Sfp1 forms aggregates under specific conditions**

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Prions in yeast act as heritable traits. The most studied prion [*PSI*<sup>+</sup>] is a heritable amyloid of the eRF3 (Sup35) protein which, in prion form, reduces stop codon readthrough accuracy. Sfp1 is a global transcriptional regulator. It is known to affect the Isp<sup>+</sup>/Isp<sup>-</sup> phenotype, which was previously thought to be caused by a prion or prion-like element.

We have shown that excess Sfp1 can lead to [*PSI*<sup>+</sup>]-dependent lethality. The lethality was accompanied by elevation in *SUP35* mRNA levels in a [*PSI*<sup>+</sup>] strain, suggesting transcriptional upregulation as the mechanism. However, *SUP45* overexpression, known to compensate for [*PSI*<sup>+</sup>]-dependent lethality caused by excess Sup35, did not affect Sfp1-derived lethality. Search for the factors that influenced the lethality of excess Sfp1 led to identification of the Hsp40 chaperone Sis1 which alleviated toxicity caused by both excess of Sfp1 and Sup35 in [*PSI*<sup>+</sup>] strains. We also showed that overproduced Sfp1 formed SDS-resistant aggregates, which colocalize with Sup35 and Sis1 in [*PSI*<sup>+</sup>] cells. Sfp1 aggregation did not depend on the presence of the [*PSI*<sup>+</sup>] or [*PIN*<sup>+</sup>] prions in the cell, but, surprisingly, it occurred only when the *SFP1* gene was expressed under the control of *CUP1* promoter. *SFP1* overexpression driven by *GPD*, *GAL1* or *SFP1* promoters did not lead to formation of Sfp1 aggregates. It is thus possible that Sfp1 aggregation occurs *in vivo* only under specific conditions which remain unknown. The work is supported by RC MCT SPbSU and by RFBR grants 16-04-00202 and 18-34-00536.

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## **P24 The response of distinct PrP fibril morphotypes to pressure**

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We provide conclusive evidence that a wide superstructural range of human PrP fibrils can be obtained by single amino acid substitutions designed to mimic several PRNP gene polymorphisms. Even if the various PrP proteins display structural homogeneity in the monomeric state, their assembly resulted into an array of distinct fibril types with distinguishable physicochemical properties.

By studying the pressure-induced dissociation kinetics of the resulting distinct PrP fibrils, we define a novel key property characterizing their assembly landscape: the fibril barostability. We report for the first time a direct correlation existing between the fibril pressure response and some of its generic properties, such as the thioflavin T binding capacities and the PK-resistance. In addition, by assuming that the system volume changes accompanying PrP fibril dissociation are at the basis of pressure effects, the study raises the possibility that the fibril type-dependent protein volume defines the prion strain phenomenon.

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## **P25 Identification and characterization of new human amyloidogenic protein PHC3**

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Self-perpetuating fibrous cross- $\beta$  aggregates (amyloids) are associated with a variety of diseases. Many amyloids possess transmissible properties, characteristic of infectious proteins (prions). There are examples of amyloids and prions playing positive biological roles. In yeast and other fungi, prions control heritable traits. Overall understanding of the scope of impact of amyloidogenic and prionogenic proteins on biological processes is only beginning to unfold.

Various *in silico* tools were designed to search for amyloidogenic proteins, although most or all of them exhibited a limited success when making *in vivo* predictions. We applied the ArchCandy algorithm capable of predicting  $\beta$ -arches to searching human proteome, followed by testing computational predictions in the yeast-based prion nucleation assay. Among other amyloidogenic proteins, we have identified short isoforms 5 and 6, encompassing the N-terminal region of the human protein PHC3. Isoforms 5 and 6 nucleate yeast prion in the absence of pre-existing prions when fused to the prion domain of the yeast protein Sup35, and form cytologically and biochemically detectable detergent resistant aggregates in the yeast cells. Aggregation properties of PHC3 isoforms are also confirmed in the bacterial expression system. PHC3 isoforms tagged by fluorescent proteins produce punctated aggregated formations when transfected into the cultured human HEK293T cells.

Full-length PHC3 protein is a component of the PRC1 complex which controls expression of homeobox genes via chromatin remodeling and plays an essential role in early embryonic development. PHC3 is thought to be involved in “clustering” of the PRC1 complexes, resulting in tight chromatin packaging and repression of transcription. Functions of short isoforms 5 and 6 remain unknown. We propose that short amyloidogenic isoforms can sequester the full-length form of the PHC3 protein into amyloid aggregates, thus downregulating its function in chromatin packaging and inducing transcription of respective genes. Such a model postulates a new two-level mechanism of epigenetic regulation of transcription. Experiments testing this model are currently underway.

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## **P26 Analysis of amyloid and prion properties of mammalian proteins using yeast assays**

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Formation of cross-beta fibrous aggregates (amyloids) is implicated in a variety of human diseases, and many amyloids have been shown to possess transmissible (prion) properties. A variety of proteins possess sequences that are potentially capable of forming amyloids. However, the molecular mechanisms of amyloid formation and propagation are difficult to investigate *in*



*vivo* due to the complexity of the human organism. Heritable endogenous amyloids found in yeast cells and termed yeast prions provide a powerful approach to the investigation of amyloidogenic properties. We have developed yeast assays for studying the prion properties of mammalian and human proteins. These assays are based on chimeric constructs, containing mammalian or human amyloidogenic proteins (or domains) fused to various fragments of the yeast prion protein Sup35. Sup35 is used as a reporter, thus enabling phenotypic detection of prion nucleation and propagation by mammalian and human amyloidogenic sequences in yeast. We have demonstrated that such chimeric constructs are applicable to studying prion properties of various proteins associated with mammalian and human diseases, including PrP, Abeta, alpha-synuclein and amylin. Mutational analyses of both PrP protein, associated with TSEs, and Abeta protein, associated with Alzheimer disease, in these constructs have identified sequences specifically influencing amyloid nucleation, and the effects of these mutations are discussed in light of structural models of PrP and Abeta amyloids. We have also infected yeast cells with Abeta amyloids produced *in vitro* and demonstrated propagation of different Abeta-based polymorphs (strains) in the yeast system. Our data show that the spectra of prion strains produced in yeast depend on the nature of amyloid-producing derivatives of Abeta. Finally, we have screened human amyloidogenic proteins, predicted by computational tools, in the yeast assays, and identified new human proteins and distinct domains with amyloidogenic properties.

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## **P27 Differential effects of chaperones and protein-sorting factors on yeast [PSI<sup>+</sup>] and [URE3] prions**

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Prions in yeast *Saccharomyces cerevisiae* are heritable genetic determinants represented by self-perpetuating protein aggregates. Yeast prions propagate in cell lineages due to tight interaction with the protein quality control (PQC) machinery, i.e. chaperones and protein-sorting factors. In our work we undertook an effort to systematically assess the effects of a set of PQC components on the most studied yeast prions, [PSI<sup>+</sup>] and [URE3]. The main PQC component that confers propagation of all known yeast amyloid prions is the hexameric ATPase Hsp104 that is required for fragmentation of amyloid fibrils. We show that, despite frequent statements in the literature, overproduction of Hsp104 cures both [PSI<sup>+</sup>] and [URE3]. On the other hand, overexpression of the SSA1 gene, which has previously been reported to destabilize [URE3], had no significant effect on its propagation in our system. We also demonstrate that overproduction of the chaperone-sorting factor Cur1 cures [URE3] while enhancing [PSI<sup>+</sup>]. We link these effects to the relocalization of the Hsp40-Sis1 into the nucleus and show that Cur1-independent changes in Sis1 localization also oppositely affect [PSI<sup>+</sup>] and [URE3] propagation. We also show that, similarly to Cur1, overproduction of the other major cytosolic Hsp40, Ydj1, enhances [PSI<sup>+</sup>]. On the basis of these observations, we propose a model for such differential effects of the Hsp40 on yeast prions. We suggest that changes in the cytosolic balance of different Hsp40, i.e. Ydj1 and Sis1, oppositely affect propagation of [PSI<sup>+</sup>] and [URE3] due to different dependence of these prions

on two distinct activities of the Hsp104 machinery, i.e. fibril fragmentation and aggregate malpartition. Our results provide new insights into the interplay between yeast prions and cellular PQC system and highlight the importance of intracellular chaperone balance for prion propagation.

Equipment of the Resource Center “Development of Molecular and Cell Technologies” at Research Park of St. Petersburg State University was used in this study. The study was supported by St. Petersburg State University (15.61.2218.2013) and grants from Russian Foundation for Basic Research (16-04-00202, 15-04-08159, 18-34-00536, and 18-34-00537), Russian Science Foundation (14-50-00069) and National Science Foundation (MCB 1516872).

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## **P28 Using zebrafish embryos to dissect PrP-dependent, SFK-mediated and A $\beta$ -induced neurotoxicity**

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Prions and amyloid  $\beta$  ( $\text{A}\beta$ ) oligomers can induce synaptic damage by binding to the cellular prion protein ( $\text{PrP}^{\text{C}}$ ) on neuronal cell surfaces. Growing evidence indicates that the ability of  $\text{PrP}^{\text{C}}$  to modulate Src family kinases (SFKs) contributes to the activation of these neurodegeneration pathways. Using gain- and loss-of-function approaches in zebrafish gastrulae, we have shown that  $\text{PrP}$ -1 -one of two  $\text{PrP}$  orthologues- supports embryonic cell adhesion by preventing the endocytosis of E-cadherin/ $\beta$ -catenin adhesion complexes via the SFKs Fyn and Yes. Remarkably, this pathway is sensitive to modulation by human  $\text{A}\beta$  peptides and resembles the mechanism by which  $\text{A}\beta$ ,  $\text{PrP}$  and SFK trigger synaptic dysfunction in mammalian models of Alzheimer's disease.  $\text{PrP}$ -2, the second zebrafish  $\text{PrP}$  orthologue, is expressed in embryonic neurons and is required for the correct development of neural structures. To clarify the molecular basis of its function, we examined the connection between neuronal  $\text{PrP}$ -2 knockdown phenotypes and SFK-controlled pathways in the CNS. Confocal analyses of spinal cord motoneurons, cranial sensory neurons and posterior lateral line ganglia in GFP transgenic embryos revealed that  $\text{PrP}$ -2 downregulation impairs the proliferation/differentiation of neural progenitors as well as the growth and navigation of axons to synaptic sites. Western blots and immunofluorescence analyses showed that these cellular defects correlate with changes in the levels of active SFKs, NMDA receptors and cell surface N-cadherin, both at cell bodies and along axonal paths and synaptic sites. These data suggest that  $\text{PrP}$ -1 and -2 share the ability to control the cell surface expression of important neuronal proteins like adhesion molecules and neuroreceptors via SFKs. To further study  $\text{A}\beta$ - and  $\text{PrP}$ -mediated, neurotoxicity, we delivered human  $\text{A}\beta$  peptides into the transparent brains of zebrafish embryos via microinjection. Confocal imaging confirmed the rapid spreading of  $\text{A}\beta$  monomers and oligomers into the developing CNS. Notably,  $\text{A}\beta$  oligomers showed a strong tendency to aggregate and accumulate within cell soma and axons, triggering axonal damage and neuronal death as well as changes in the levels of active SFKs, NMDA, AChR and surface N-cadherin, reminiscent of the effects observed upon  $\text{PrP}$ -2 downregulation. These results suggest that  $\text{PrP}$ -2- and  $\text{A}\beta$ -mediated neurotoxicity converge at SFK target pathways. Finally, we have established an automated methodology to track and record changes in motor (swimming) behavior of zebrafish larvae upon  $\text{PrP}$  gain- and loss-of-function and/or exposure to neurotoxic  $\text{A}\beta$  oligomers. These experiments provide a functional correlate to our cellular and molecular analyses, and set the stage for an integrative screening approach to search for potential anti-neurodegeneration compounds using morphological, behavioral, cellular and molecular readouts. Supported by INNOVATE Contrato 168-PNICP-PIAP-2015.

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## **P29 Neuroanatomical and behavioural tools to study PrP-mediated neurotoxicity in zebrafish embryos**

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Using genetic approaches in zebrafish embryos, our laboratory has experimentally addressed some of the cellular and molecular events controlled by PrP<sup>C</sup>-mediated signaling in vivo and their connection to neurotoxic pathways. In particular, we have been studying the role of PrP as a modulator of cell adhesion complex and neuroreceptor trafficking. These experiments benefit greatly from the use of high resolution fluorescent imaging techniques to visualize the localization of proteins of interest in externally transparent and rapidly developing embryos. Assessing the physiological relevance of these findings requires the use of additional cell markers to reliably identify diverse neuroanatomical structures and specific cell populations in the embryonic CNS. To this aim, we surveyed the localization patterns of a panel of primary antibodies against mammalian and zebrafish neuronal proteins via whole-mount immunostaining and confocal microscopy in wildtype and GFP-transgenic zebrafish embryos. The resulting images provide a comprehensive, high-resolution, three dimensional overview of various groups of embryonic motor and sensory neurons, making it possible to evaluate how the development of their cell bodies and axons may be controlled by PrP. Hence, we used these neuroanatomical markers to characterize the morphological defects caused by morpholino downregulation of PrP-2, the zebrafish prion protein orthologue expressed in embryonic neurons. To evaluate the impact of PrP-2-dependent developmental defects on the neuronal physiology of these fish larvae, we conducted motor behavioral analyses using a multi-well-based methodology in an automated tracking system (Zebralab). Swimming behavior data (speed, distance, trajectories) under normal conditions and upon light stimulation could thus be potentially linked to defects in discrete neuroanatomical structures, giving us the possibility to focus our genetic, cellular and molecular analyses of PrP function on specific subsets of neurons.

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## **P30 Effects of mutations, polymorphisms, and other variants of the PRND gene on the PrP<sup>C</sup> structure from MD simulations analyzed with essential collective dynamics and machine learning methods**

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## **P31 Isolation and characterization of strain-specific prion particles**

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There is a huge gap in our knowledge of prion disease: which prion particle is most pathogenic? Given that prion aggregation is at the very heart of prion disease, it is vital that we understand how the aggregation translates into pathology and which particle is most responsible. Treatments that focus on removing or blocking prion particles are hindered without this information.

For many years, large protease resistant prion protein aggregates have been studied. Their structural stability and insolubility make them easy to purify in a proper yield for *in vitro* and *in vivo* studies. In recent years, smaller protease sensitive prion particles have been identified and shown to have an important role in the disease. However, very little is known about their size, structure, infectivity and neurotoxicity properties. Their low concentration and structural instability make their isolation challenging. The most detailed characterization of these small prion particles was reached by using Asymmetric Flow-Field Flow Fractionation (AF<sub>4</sub>), a technique well suited for the separation of macromolecules based on size (Silveira et al. *Nature* 437, 257-261, 2005).

In this work, we use the same technique to assess strain-specific oligomer distributions and seeding activities.

Brain homogenates from mouse-adapted scrapie, 22L and ME7, hamster-adapted scrapie 263K and Hyper, and mouse-adapted CJD, fCJD (E200K) and sCJD, were incubated under mild detergent conditions to solubilize the protein content while preserving the natural oligomeric forms. AF<sub>4</sub> was used to separate aggregates based on size and the presence of PrP in each fraction was evaluated by immunoblot. The hydrodynamic radius (Rh) of the particles present in each fraction was determined by dynamic light scattering. By calculating the proportion of PrP particles in size groups ranging from 3 to ~100nm, we have generated PrP oligomer Rh distribution plots for each prion strain. PK digests of the fractions allows us to define which oligomers are more or less protease sensitive.

Our goal is to isolate and characterize prion oligomers from many different strains in order to both identify any strain-specific pathogenic species and better focus therapeutic strategies that target misfolded oligomers of PrP.

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## **P32 Role of ovine Gln171 and surrounding residues in the PrP<sup>C</sup>-PrP<sup>res</sup> conversion: A preliminary study**

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Scrapie is a prion disease in sheep that requires both prion exposure and genetic susceptibility. Susceptibility is defined by amino acid polymorphisms in the ovine prion protein gene Prnp, particularly at positions 136, 154 and 171 (Goldmann et al., 2005). However an increasing number of polymorphisms are being described currently suspected to trigger similar effects in the resistance/susceptibility against transmissible spongiform encephalopathies in small ruminants.

Although most susceptible animals are those with genotype V<sup>136</sup>R<sup>154</sup>Q<sup>171</sup>/ V<sup>136</sup>R<sup>154</sup>Q<sup>171</sup> (VRQ/VRQ), in this context, sheep carrying genotype A<sup>136</sup>R<sup>154</sup>Q<sup>171</sup>/ A<sup>136</sup>R<sup>154</sup>Q<sup>171</sup> (ARQ/ARQ) are

susceptible to Scrapie whereas those with genotype A<sup>136</sup>R<sup>154</sup>R<sup>171</sup>/ A<sup>136</sup>R<sup>154</sup>R<sup>171</sup> (ARR/ARR) are resistant. In other words, this naturally occurring Q171R polymorphic variant of PrP renders sheep resistant to Scrapie and BSE, suggesting that the residue present at this position is of paramount importance in modulating the degree of susceptibility to disease. Despite them, R171 have been found in most atypical Scrapie affected animals, other variant of the disease postulated as probably sporadic which incidence is usually one case for each outbreak.

Protein Misfolding Cyclic Amplification (PMCA) technique mimics the prion replication *in vitro* with a similar efficiency to the *in vivo* process but with accelerated kinetics (Fernández-Borges et al., 2009). The recent development of the recombinant-PMCA (rec-PMCA) has permitted to change the source of PrP<sup>C</sup> from brains to recombinant protein, allowing a fast, easy and cheap way to study mutations that affect the conversion process, the species barrier or structural characteristics of the PrP<sup>res</sup> among others (Fernández-Borges et al., 2017).

The main goal of this study is to assess the role of the ovine PrP amino acid located at position 171 and its structurally surrounding residues in the PrP<sup>C</sup> to PrP<sup>res</sup> conversion.

According to several structures described for ovine prion protein (Eghiaian et al., 2004; Haire et al., 2004, Lysek et al., 2005) Gln171 is located in the PrP surface exposed to the solvent in the loop between beta strand-2 and alpha helix-2. Due to that, Arg171 could have a strong electrostatic effect in the charge distribution in this region where other negative amino acids are located, as Asp170 and Asp181.

A series of rec-PMCA experiments were designed to check the effect of the charge, size and/or hydrophobicity of distinct residues in this polymorphic area as well as the thermal stability for residue 171 mutants.

Preliminary results suggest the significant impact of these physicochemical properties of residues on the conversion of PrP<sup>C</sup> to PrP<sup>res</sup>, launching a new methodology to shed light over the prion propagation phenomenon.

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### **P33 Analysis of amyloid properties of the NSF protein forming detergent-resistant aggregates in the brain of rat *Rattus norvegicus***

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Amyloids are fibrillar protein aggregates with cross-beta structure. Pathological accumulation of amyloid aggregates can accompany socially important neurodegenerative disorders like Alzheimer disease which is linked with accumulation of Aβ peptide or Parkinson disease associated with aggregation of α-synuclein protein. Amyloidosis study is a top priority of the modern science. Moreover, the data appeared about the existence of the functional amyloids which play essential roles in the regulation of vital processes in a wide range of organisms. Until recently there were no universal methods for proteomic screening of amyloids therefore we can assume that our knowledge about pathological and functional amyloids are very limited.

Recently we've developed a novel method of proteomic screening named PSIA-LC-MALDI that allows us to search for proteins forming SDS-resistant amyloid-like aggregates in different organisms. The amyloid properties of the proteins identified in such screenings should be confirmed by further individual analysis. Thereby we can detect new still not characterized functional and pathological amyloids in different organisms. By using this method we can expand our knowledge about amyloids and their functions.

Through this technique we've analyzed the brain of young males of *R. norvegicus*. Among a wide range of identified proteins capable of forming detergent-resistant aggregates we selected the proteins which are more suitable for the role of functional amyloids. The NSF protein was the one that caught our attention. NSF (N-ethylmaleimide-sensitive fusion protein) is required for vesicle-mediated transport i.e. the process underlying the neurotransmission. The purpose of this research is the analysis of amyloid properties of NSF protein.

Basing on the result of semi-denaturing detergent agarose gel electrophoresis (SDD-AGE) of total brain lysate, we conclude that NSF forms detergent-insoluble aggregates. This result serves as a confirmation of NSF detergent-resistance - the basic feature of amyloid fibrils. Also NSF has two potential amyloidogenic regions identified by ArchCandy algorithm: 122-140 and 350-367 aa. In order to prove aggregation-prone properties of NSF *in vivo* we've carried out heterologous production of protein containing NSF(117-744aa) fragment bearing amyloidogenic regions fused with fluorescent protein YFP in *Saccharomyces cerevisiae*. The chimeric gene *NSF(117-744)-YFP* was expressed under the control of *CUP1* promoter in the yeast strain GT409. As a result NSF(117-744)-YFP in yeast cells forms aggregates presented with two different morphologies: dots and ribbons. These structures are similar to well-known heterologous amyloid aggregates formed in *S. cerevisiae*.

Taking into consideration the detergent-resistance of NSF and its propensity to form aggregates we assume that this protein has amyloid properties *in vivo*. This leads us to conclusion that the NSF protein can be considered as a novel functional amyloid.

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#### **P34 Solid State NMR Spectroscopy: Investigation of the core arrangement in the prion domain of Sup35NM S17R mutant**

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The [PSI<sup>+</sup>] prion is a self-propagating amyloid form of Sup35 protein, which is a subunit of the translation termination factor [1]. Aggregation and the subsequently decreased level of soluble Sup35 lead to more frequent read-through of premature termination codons. Sup35 includes an N-terminal domain (N), which is both necessary and sufficient for prion propagation, a charged domain (M) which increases the solubility of the protein and the C-terminal translation termination domain [2]. Despite the high interest on Sup35NM (N-domain together with the M-domain), there is no consensus on the organization of monomers within Sup35NM fibrils [3]. One single point mutation, the substitution of S17 by a positively charged R residue, is sufficient to prevent Sup35pS17R monomers from being co-fibrillated with Wild-type Sup35p [4]. However, the S17R monomers can form fibrils themselves at 4°C, as well as at 37°C.

We have used symmetry-based constant-time homonuclear dipolar recoupling [5] for distance measurements between backbone  $^{13}\text{CO}$ - $^{13}\text{CO}$  in amino-acid selectively  $^{13}\text{CO}$  labeled samples of WT and S17R Sup35pNM. Our ssNMR results showed that different regions of the protein for WT and the S17R mutant of Sup35NM are involved in the amyloid core region [6].

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### **P35 RNA modulates aggregation of the murine recombinant prion protein and promotes oligomeric seed formation**

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By employing RNAs of various source and size, we have studied the role of RNA in the aggregation of murine recombinant prion protein (rPrP<sup>23-231</sup>) using light scattering, fast-kinetics in stopped-flow, co-sedimentation, transmission electron microscopy (TEM), circular dichroism (CD) and isothermal titration calorimetry (ITC). Our results show that RNA modulates rPrP<sup>23-231</sup> aggregation in a concentration dependent manner. At a low RNA to protein ratio (e.g. 1: 100), RNA stimulates mostly amorphous aggregation of rPrP<sup>23-231</sup>. In contrast, at higher RNA to protein ratio (1:1), RNA inhibits aggregation of rPrP<sup>23-231</sup>, and instead, facilitates formation of the small oligomeric species capable of enhancing *de novo* aggregation of rPrP<sup>23-231</sup>. Furthermore, RNA co-sediments with rPrP<sup>23-231</sup> aggregates in an RNase resistant manner indicating a direct interaction between the two. Sequence analysis suggests that the interaction is not specific to any RNA sequence; this calls for a more fundamental explanation for RNAs role in rPrP<sup>23-231</sup> aggregation. Our study, together with the earlier reports (1-3), suggests that the direct modulatory roles of RNA affecting aggregation pattern of the model prion proteins are of physiological relevance (4).

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### **P36 Dicer knock-down attenuates aberrant prion protein accumulation in a cellular model of prion diseases**

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**Introduction:** Prion diseases are fatal neurodegenerative disorders entailing the template driven propagation of an aberrant and partially resistant to Proteinase K (PK) prion protein isoform (PrP<sup>Sc</sup>), which accumulates as a result of a conformational conversion imposed on the normal PrP<sup>C</sup> protein. Little is known on the precise molecular mechanisms and/or molecules associated with prion diseases promotion/progression. Recent studies highlight microRNAs (miRNAs) involvement in prion diseases (Llorens et al. 2018, *PLoS pathogens*, 14(1): e1006802), and indicate that Dicer, a key molecule in miRNA maturation, is detected in PrP<sup>C</sup> associated complexes (Gibbings et al. 2012, *Nat Struct Mol Biol*, 19(5):517-524), suggesting a role for Dicer in prion diseases.

**Aims:** We sought to investigate Dicer's involvement in prion propagation, using an *in vitro* disease cellular model and RNA interference technology for Dicer knock-down.



**Materials and Methods:** We utilized murine neuroblastoma cells persistently infected with the 22L scrapie strain (22LN2a#58, scN2a) and the corresponding non-prion infected cell line (N2a#58, N2a). Dicer was transiently knocked-down with three independent siRNAs (siI, siA, siB). Efficient Dicer knock-down was confirmed at both the mRNA and protein levels (qRT-PCR and Western Blot analysis) and led to a significant reduction of a panel of mature miRNAs. Prion propagation was assessed via estimation of aberrant PrP accumulation, measured as the relative conversion of endogenous PrP to the proteinase K resistant PrP<sup>Sc</sup>. Relative PrP conversion for each sample (PrP<sup>Sc</sup>/PrP<sup>total</sup>) was determined by densitometric analysis on 6H4 probed Western Blots (WB) of PK-treated (PrP<sup>Sc</sup>) and non PK-treated (PrP<sup>total</sup>) cell lysates and was expressed relative to the conversion of the negative control (scrambled-siRNA, not recognizing any sequence in the murine transcriptome).

**Results:** Significant reduction of Dicer mRNA (siI: 66%, siA: 82%, siB: 85%) and protein levels (siI: 20%, siA: 46%, siB: 23%) was observed at 48 and 72 hours after transfection respectively relative to negative controls (two independent experiments for each siRNA). Reduced PrP<sup>Sc</sup> accumulation relative to negative controls was detected at 72 hours after transfection for all siRNAs (siI: 60%, siA: 68%, siB: 58%), while PrP mRNA levels did not show similar reduction. Dicer levels and PrP<sup>Sc</sup> accumulation were correlated.

**Discussion:** Our preliminary data indicate that Dicer knock-down leads to reduction of PrP<sup>Sc</sup> accumulation in scN2a cells (~50%) without similarly affecting PrP mRNA levels. Further analyses are underway to precisely identify Dicer's involvement (affected miRNAs and their targets, Dicer-PrP<sup>C</sup>/PrP<sup>Sc</sup> interactions) in prion propagation in the utilized disease model.

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### **P37 Five Prion-curing systems in *Saccharomyces cerevisiae*: Upf proteins, inositol polyphosphates, Btn2p, Cur1p, and Hsp104p**

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### **P38 DNP-Enhanced solid-state NMR at Cryogenic Temperatures: a Tool to Snapshot Conformational Ensembles of $\alpha$ -Synuclein in Different States**

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**P39 Interference of a D-enantiomeric peptide with hetero-association of amyloid- $\beta$  oligomers and prion**

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder that affects millions of people worldwide. One hallmark of the disease is the aggregation of amyloid- $\beta$  (A $\beta$ ) into soluble oligomers and insoluble fibrils. Several studies indicate that oligomers rather than fibrils are the most toxic species in disease propagation. A $\beta$  oligomers bind with high affinity to membrane-associated prion protein (PrP), leading to toxic signalling across the cell membrane, which makes this interaction an attractive therapeutic target. Here, we studied the A $\beta$ -PrP interaction in detail. Our data show that full-length, soluble human (hu) PrP(23-230), as well as huPrP(23-144) lacking the globular C-terminal domain, bind to A $\beta$  oligomers to form megadalton-sized complexes. Following purification by sucrose density gradient ultracentrifugation, the A $\beta$  and huPrP contents in these hetero-assemblies were quantified by RP-HPLC. The A $\beta$ /huPrP molar ratio in the assemblies showed some variation in a narrow range depending on the molar ratio of the initial mixture. Specifically, the molar ratio in the assemblies of about 4 A $\beta$  to 1 huPrP in presence of an excess of huPrP(23-230) or huPrP(23-144) suggests that four A $\beta$  units are required to form one huPrP binding site. The A $\beta$ -binding all-D-enantiomeric peptide RD2D3 competed with huPrP for A $\beta$  oligomers in a concentration-dependent manner. Our results highlight the importance of A $\beta$  oligomer-specific multivalent interactions and demonstrate that compounds can be identified, which compete with PrP for these interactions.

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**P40 The universal method of proteomic screening for amyloid proteins leads to the discovery of the novel functional amyloids of mammalian brain and *Saccharomyces cerevisiae* cell.**

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Amyloids are non-branching fibrils that are composed of stacked monomers stabilized by intermolecular  $\beta$ -sheets. Until recently there were no methods for large-scale proteomic screening for amyloids, which made the discovery of each new amyloid a notable scientific event. Using

our novel proteomic approach called PSIA-LC-MALDI, we have screened the proteomes of *R. norvegicus* brain and *S. cerevisiae* cell and identified several amyloid-like proteins. This method is based on the resistance of amyloid aggregates to treatment with SDS that allows the separation of amyloids from the majority of other protein complexes of a non-amyloid nature.

Basing on the results of the screen of yeast proteome, we created the list of the proteins forming SDS-resistant aggregates and containing amyloidogenic regions predicted *in silico*. The majority of identified proteins are associated with the cell-wall. One of the proteins identified – Toh1, is the subject of special interest. We showed that the Toh1-YFP hybrid protein forms fluorescent detergent-resistant aggregates in yeast cell while being expressed under its own *P<sub>TOH1</sub>* or inducible *P<sub>CUP1</sub>* promoter. We demonstrated that Toh1 amyloidogenic region 1-163 aa. binds Congo Red and manifest “apple-green” birefringence when examined between crossed polarizers in bacterial C-DAG system. Furthermore, Toh1-YFP exhibits the dependency of aggregation rate on the presence of the yeast prions: [*PSI*<sup>+</sup>] and [*PIN*<sup>+</sup>], and is co-localized with a high frequency with Sup35NM-CFP aggregates. All these data show that amyloid-like aggregation of non-Q/N-rich protein Toh1 depends on its interaction with Q/N-rich prions.

Moreover, several proteins forming SDS-resistant aggregates and containing amyloidogenic regions were identified in rat brain. Among them was the FXR1 protein that regulates translation of target mRNAs and takes part in controlling memory and emotions. We showed that N-terminal region of the FXR1 protein underlies the ability of full-length FXR1 to form SDS-resistant oligomers and insoluble aggregates in the brains of young and healthy rat males and forms amyloid fibrils in bacterial C-DAG system and *in vitro*. Furthermore, we demonstrated that FXR1 forms amyloid conformers that strongly co-localize with thioflavine S, bind RNA and prevents its RNase-mediated degradation in the cortex neurons of the rat brain. *In vitro* assay allowed us to confirm the ability of amyloid conformers of the N-terminal region of FXR1 to bind poly(A)-RNA extracted from the rat brain. Interestingly, the N-terminal region of FXR1 is highly conserved across mammals and contains identically arranged potentially amyloidogenic sequences in evolutionary distinct vertebrates. These data support our hypothesis that the ability of FXR1 amyloid conformers to protect specific mRNAs from degradation is conserved in evolution.

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#### **P41 DLP1-dependent mitochondrial fragmentation and redistribution mediate prion-associated mitochondrial dysfunction and neuronal death**

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Mitochondrial malfunction is a universal and critical step in the pathogenesis of many neurodegenerative diseases including prion diseases. Dynamin-like protein 1 (DLP1) is one of the key regulators of mitochondrial fission. We investigated the role of DLP1 in mitochondrial fragmentation and dysfunction in neurons using *in vitro* and *in vivo* prion disease models. Mitochondria became fragmented and redistributed from axon to soma, correlated with increased mitochondrial DLP1 expression in murine primary neurons (N2a cells) treated with the prion peptide PrP<sup>Sc</sup>106–126 *in vitro* as well as in prion strain-infected hamster brain *in vivo*. Suppression of DLP1 expression by DLP1 RNAi inhibited prion-induced mitochondrial fragmentation and dysfunction (measured by ADP/ATP ratio, mitochondrial membrane potential, and mitochondrial integrity). We also demonstrated that DLP1 RNAi is neuroprotective against prion peptide in N2a

cells as shown by improved cell viability and decreased apoptosis markers, phosphorylated-caspase 3 induced by PrP106–126. On the contrary, overexpression of DLP1 exacerbated mitochondrial dysfunction and cell death. Moreover, inhibition of DLP1 expression ameliorated PrP106–126-induced neurite loss and synaptic abnormalities (i.e., loss of dendritic spine and PSD-95, a postsynaptic scaffolding protein as a marker of synaptic plasticity) in primary neurons, suggesting that altered DLP1 expression and mitochondrial fragmentation are upstream events that mediate PrP106–126-induced neuron loss and degeneration. Our findings suggest that DLP1-dependent mitochondrial fragmentation and redistribution plays a pivotal role in PrPSc-associated mitochondria dysfunction and neuron apoptosis. Inhibition of DLP1 may be a novel and effective strategy in the prevention and treatment of prion diseases.

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**P42 A computational workflow to investigate the molecular interaction properties and aggregation propensity of prion proteins.**

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The amyloid conversion is a massive  $\alpha \rightarrow \beta$  transition affecting a folded protein structure in response to physical or chemical stimuli and characterizing a plethora of diseases [1]. The investigation of mutants, structurally similar to their native isoform but inherently prone to amyloid conversion, may be a viable strategy to elucidate the structural features connected with amyloidogenesis [2]. We have recently designed a computational workflow based on the combination of molecular dynamics (MD) and grid-based approaches suited for the pairwise comparison of closely related protein structures [3]. This method was applied on the cellular prion protein (PrPC) as a case study to address the qualitative/quantification of the structural features conferred by either E200K mutation or by the treatment with  $\text{CaCl}_2$ , both known to induce a pathogenic conversion of this protein. The aggregation propensity of PrP protein systems including wild type, wild type treated at different  $[\text{Ca}^{2+}]$  and E200K mutant has been specifically investigated by using the above mentioned computational approach [4]. Plausible models for the self-assembly of either the E200K mutated or  $\text{Ca}^{2+}$ -bound PrPC have been provided together with structure-based information and new prompts that might feed future experimental or computational studies on PrPC aggregation. In particular, a plausible 3D structure of PrP-E200K dimer was drawn and investigated in detail by the combination of classical molecular dynamics (MD) with fragment molecular orbital (FMO) approach [5]. Such an approach provided not only for corroboration to the aggregation hypothesis but also allowed to estimate the contribution of single protein residues to the protein-protein interaction energy, i.e. residues with a higher impact to the stability of the dimer.

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**P43 Prion protein conformational landscape studied by mass spectrometry and ion mobility**

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See WS7

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## **II. Animal prion disease**

**P44 Prion like protein gene (*PRND*) polymorphisms associated with scrapie susceptibility in Korean native black goats**

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The polymorphisms of the prion protein (*PRNP*) gene, which encodes normal prion proteins (PrP), are known to be involved in the susceptibility of prion diseases. The prion-like protein (doppel) gene (*PRND*) is the paralog of the *PRNP* gene and is closely located downstream of the *PRNP* gene. In addition, the polymorphisms of *PRND* correlate with disease susceptibility in several animals. The goal of this study was to examine the genetic characteristics of the *PRND* gene and to evaluate the susceptibility to scrapie in Korean native black goats. We analyzed the genotype and allele frequencies of *PRND* polymorphisms in 246 Korean native black goats and found a total of six single nucleotide polymorphisms (SNPs) within the open reading frame (ORF) of the *PRND* gene. Among them, we identified one novel SNP, c.99C>T. Allele frequencies of the *PRND* c.28T>C polymorphism are not significantly different between healthy Korean goats and scrapie-affected goats ( $P = 0.717$ ). In addition, the genotype distributions of the *PRNP* p.H143R according to genotype and allele distributions of the *PRND* c.28T>C was significantly different ( $P < 0.0001$ ). Finally, using PolyPhen-2 and PROVEAN, we predicted that two non-synonymous SNPs, c.65C>T and c.286G>A, in the *PRND* gene can have a detrimental effect on doppel. We first reported genetic polymorphisms of the *PRND* gene in Korean native black goats. According to the genetic distribution of *PRND* c.28T>C, Korean native black goats showed a genetic distribution of the *PRND* gene that is comparatively susceptible to scrapie.

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**P45 Regional genetic selection plan for the knowledge of PRNP genotype distribution of the Sicilian flocks, 2013-2017**

MacriD (1), Bivona M (1), Lo Mascolo F (1), Messina G (1), Chiarenza G (1), Migliore S (1), Buttitta O (1), Vitale F (1) and Stancanelli A (1)

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The Sicilian genetic selection plan for Scrapie originated from the first national one, launched in December 2004. Objective of that plan was to increase the frequency of genetic resistance characters to the TSEs in the ovine population in order to: contribute to the eradication of the sheep TSE, to the creation of 'low risk' flocks of TSE and to the protection of human and animal health. The increase in resistance characters is achieved through: elimination of the VRQ allele by prohibiting the use of reproducers carrying such allele; increase in the frequency of the ARR allele in the farms participating in the plan; establishment of stocks of resistant homozygous rams (ARR/ARR), also useful for restocking infected farms; progressive decrease in the frequency of the ARQ allele in the farms adhering to the plan. That plan, however, presented a limitation for its complete realization, in fact the adherence to it was on a voluntary basis, mandatory adherence was only for flocks of high genetic merit. So voluntary adherence caused low participation by breeders, limiting the progress towards achieving resistance characteristics in the national sheep population. According to the national plan, in Sicily from 2004 to 2013 were genotyped about 2.600 animals. In January 2013, finally, was launched the first regional genetic selection plan, implemented perfectly in accordance with the national plan with a single exception: adherence was compulsory for commercial farms with more than 200 sheep. From January 2013 to December 2017 a total of 48.000 samples, belonging only to the male line as required by the selection plan, were conferred to the laboratory. Based on the success of the first plan, in 2015 started the second genetic selection plan which became obligatory for all sheep farms, so it was possible to investigate the Sicilian sheep heritage. The genotypes obtained were classified, according to the decree of 25 December 2015, in sensitive and resistant animals. Resistant population, in which at least one ARR allele is present, was 61.35%; instead the susceptible one was 38.65%, with presence of VRQ allele in 4.22%. The monitoring activity of scrapie showed a high spread of the disease. In fact in Sicily from 2013 to 2017 were found 28 new outbreaks of SCRAPIE. Distribution of resistant genotypes in the territory is homogeneous and higher than 50% of the tested population. No increase of the frequency of the ARR allele was highlighted during these years, probably it will be assessed in the next years, as an expected result of the correct application of the regional plan.

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**P46 Low doses of a bioherbicide favors prion aggregation and propagation in vivo**

Lafon PA (1), Imberdis T (1,2), Wang Y (1,3), Torrent J (1), Robitzer M (4), Huetter E (1), Alvarez-Martinez MT (5), Chevallier N (1), Givalois L (1), Desrumaux C (1), Liu J (3) and Perrier V (1)

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Pesticides are organic or synthetic chemicals used to preserve agricultural areas, to improve yields, as well as the preservation of food products. The massive use of pesticides causes environmental pollution and food contamination leading to health problems. Epidemiological studies in humans have shown that two pesticides, rotenone and paraquat, could influence the risk of developing Parkinson's disease in a population exposed to high and/or frequent doses of these pesticides. Recently, Richardson et al. (2014) showed that in some patients with Alzheimer's disease possessing the ApoE4 allele, the amount of DDE (DDT metabolite) in plasma was 3.8 times higher than in healthy people. A more general role of pesticides in neurodegenerative disorders is now suspected.

In our laboratory, new molecules that interact with prion protein and promoting the formation of PrP<sup>Sc</sup> oligomers were identified (Ayrolles-Torro et al., 2011). One of these compounds, A6, described for its herbicidal properties, belongs to the family of  $\alpha$ -terthienyls synthesized by plants such as marigolds.  $\alpha$ -terthienyls' toxicity is due to the formation of free radicals. Recently, we showed that A6 decreases zebrafish larvae survival. The compound accumulates in neurons at submicromolar concentrations leading to neurodegeneration (Nasri et al., 2016). Here, we studied the effect of the A6 compound in a mouse model of prion disease. This molecule was injected intra-peritoneally at several concentrations: 5, 10 and 20 mg/kg. Our results showed that at 5 mg/kg, A6 accelerates the onset of the pathology, in correlation with an increase of amyloid deposits in the brain, as well as astrocytic gliosis and spongiosis. However, at 10 and 20 mg/kg, the treatment decreases the histopathological signs of prion disease. A rapid centrifugation assay developed in the team (Imberdis et al., 2016), showed that A6 compound from 1mM and above induces prion aggregation *in vitro*. Conversely, at lower concentrations, A6 does not produce prion aggregation but promotes soluble oligomers, which are known to be neurotoxic. These results are consistent with our *in vivo* study and suggest that at high concentrations, A6 creates non-toxic aggregates trapping prion replication and at lower concentrations, A6 rather promotes neurotoxic oligomers.

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#### **P47 Plasma cholesterol level determines *in vivo* prion propagation**

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Transmissible spongiform encephalopathies (TSE) are fatal neurodegenerative diseases with an urgent need for therapeutic and prophylactic strategies. At the time when the blood-mediated transmission of prions was demonstrated, *in vitro* studies indicated a high binding affinity of the scrapie prion protein (PrP<sup>Sc</sup>) with apolipoprotein B-containing lipoproteins, i.e. the main carriers of cholesterol in human blood (Safar et al., 2006). The aim of the present study was to explore

the relationship between circulating cholesterol-containing lipoproteins and the pathogenicity of prions *in vivo*. We showed that in mice with a genetically-engineered deficiency for the plasma lipid transporter phospholipid transfer protein (PLTP), abnormally low circulating cholesterol concentrations were associated with a significant prolongation of survival time after intraperitoneal inoculation of the 22L prion strain. Moreover, when circulating cholesterol levels rose after feeding PLTP-deficient mice a lipid-enriched diet, a significant reduction in survival time of mice together with a marked increase in the accumulation rate of PrP<sup>Sc</sup> deposits in their brain were observed (Perrier et al., 2017). Our results suggest that the circulating cholesterol level is a determinant of prion propagation *in vivo*, and that cholesterol lowering strategies might be a successful therapeutic approach for patients suffering from prion diseases.

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#### **P48 Stereotaxic delivery of M1000 prions to the hippocampal CA1 region of wild type mice produces acute prion neurotoxicity**

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Prion diseases are fatal neurodegenerative disorders that include Creutzfeldt–Jakob disease in humans, as well as scrapie in sheep and bovine spongiform encephalopathy in cattle. Similar to other more common neurodegenerative diseases such as Alzheimer's and Parkinson's disease, prion diseases are also characterized by abnormal protein deposits in the brain, which occur due to misfolding of the normal prion protein (PrP<sup>C</sup>) into disease-associated conformers (PrP<sup>Sc</sup>) through an incompletely defined pathway. Correlating with the presence of PrP<sup>Sc</sup>, synaptic dysfunction in animal models of prion disease appears to be an early and fundamental pathogenic event albeit occurring through unresolved mechanisms. To facilitate redressing our rudimentary understanding of the pathophysiology of synaptic dysfunction and consequent behavioural disturbances, biologically relevant and tractable *in vitro* and *in vivo* models are required.

The goal of this project is to further develop and utilize our *in vivo* model of prion acute neurotoxicity to better understand the pathophysiology of acute hippocampal synaptic dysfunction through use of behavioural and subsequent biochemical and morphological analyses. M1000 prions are mouse-adapted from Gerstmann-Sträussler-Scheinker syndrome (GSS). Utilising stereotactic surgery, ~11-week-old female wild-type C57BL/6 mice were bilaterally injected with 4µl of either 10% (w/v) M1000 brain homogenate (from terminally sick mice) or normal brain homogenate (NBH) into the CA1 region of each hippocampus (coordinates -2.5mm from bregma, +/- 2.5mm laterally and -1.5mm depth) (n=10 per group). Seven days following stereotaxic injection, the mice commenced a series of motoric, behavioural/ethological and cognitive testing, which included Rotarod, Y-maze, Open Field, conditioned fear memory and the Barnes Maze. Following stereotaxic injection (fsi), open field (day 7 fsi) and Rotarod (day 10 fsi) testing showed no significant inter-group differences in gross motor performance between M1000 and NBH injected groups. One hour Y-maze (day 8 fsi) showed significant impairment in the recognition of the novel arm in the first 30 and 60 seconds of testing. Conditioned fear memory testing (day 8 fsi) showed a significant increase in freezing in M1000 exposed mice 24 hours following successful auditory stimulus-foot shock pairing. Biochemical and immunohistochemical analysis of key synaptic proteins at different time-points fsi confirmed altered protein levels in the hippocampus of M1000 injected mice, with comparisons also made to changes found at the terminal stage of prion disease.



Collectively, these data illustrate the ongoing development of a novel acute *in vivo* prion toxicity model, which will allow further interrogation of pathogenic mechanisms related to synaptic dysfunction and disturbed behaviour and cognition in prion disease.

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#### **P49    Detection PrP<sup>CWD</sup> in body fluids of CWD-affected cervids by RT-QUIC**

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**Aims:** Chronic wasting disease(CWD) is the only prion disease affecting free-ranging animals, reported in North America, South Korea, Norway and Finland. Differing from other prion diseases, CWD agents(PrP<sup>CWD</sup>) are shed in blood, saliva, urine and feces, which most likely contributes to the relatively efficient horizontal transmission between cervids species. The development of amplification-based seeding assays has been instrumental in the detection of low levels of prions in clinical samples. Using NaPTA precipitation and/or real-time quaking-induced conversion(NaPTA/RT-QUIC), we established an ultrasensitive detection method for PrPCWD in the cerebrospinal fluid, saliva, and urine of CWD affected cervids. In addition, we performed mouse bioassay to assess PrPCWD infectivity in cervid biological fluids(saliva, urine and CSF) using transgenic mice overexpressing elk prion protein (TgElk mice).

**Methods:** Five saliva, two urine and two CSF from CWD-infected cervids at the terminal stage of disease, and five saliva samples from normal cervids were stored at -80°C. 100uL of the saliva or urine samples were mixed with 10uL 2.8% sodium phosphotungstic acid and incubated for 1hr at 37°C with shaking at 1,350rpm. Samples were centrifuged for 30min at 16,100g. The pellet was resuspended in 10uL of 0.1% SDS/PBS for 30min at 55°C. RT-QUIC reactions were set up in 96-well clear bottom optical plates and consisted of 98uL RT-QUIC buffer(final concentrations of 1XPBS, 1mM EDTA, 10uM Thioflavin, 300mM NaCl), 0.1mg/ml Syrian hamster recombinant protein(23-231) and 2uL of sample. The RT-QUIC assay was performed on a FLUOstar Omega fluorescence plate reader that was preheated to 55°C for 60hr with 1min shaking at 700rpm followed by 1min incubation. For CSF, the RT-QUIC reaction consisted of 93uL RT-QUIC buffers and 7uL of sample. The plate reader was preheated to 42°C and the other reaction conditions were same. For bioassay, 18 mice(6 mice per group, 8-week-old) were inoculated intracranially with 20uL of each inoculums (saliva, urine, CSF).

**Results:** PrP<sup>CWD</sup> was detected in terminal urine, saliva and CSF samples from two sika deer (experimental cases), and terminal saliva samples from three cervids were also observed to consistently yield positive results by the RT-QUIC assay. All saliva samples from normal cervids were determined to be negative based on assay threshold values(>50,000). The survival time of CSF inoculated mice was 231±37dpi and attack rate was 100%(6/6). Saliva and urine inoculated mice remain alive at 300dpi and the infectivity of these inocula remain to be determined.

**Conclusions:** We demonstrate that CWD prions can be detected by NaPTA/RT-QUIC in the saliva and urine, and by RT-QUIC alone in the CSF of red deer and sika deer at terminal stages of disease, which suggesting that this is a useful technique to assist in the diagnosis and surveillance of CWD.

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## **P50 Prion seeding amplification in CWD-affected elk brain**

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**Aims:** Chronic wasting disease (CWD) is the only prion disease affecting free-ranging animals, reported in North America, South Korea, Norway and Finland. The enzyme-linked immunosorbent assay(ELISA), western blotting(WB), immunohistochemistry(IHC) and bioassay have been used in prion disease diagnosis and surveillance. The developments of amplification-based seeding assays have been instrumental in the detection of low levels of prions in preclinical samples. Using protein misfolding cyclic amplification with beads(PMCAB) and real-time quaking-induced conversion (RT-QUIC) and bioassay, we compared sensitivity of detection methods for PrP<sup>CWD</sup> in CWD affected elk brain.

**Methods:** A brain homogenate prepared from the pool of two clinical elk CWD cases in 2001(E190Y+229Y) in Korea and were stored at -80°C. For end-point titration, six TgElk mice were inoculated intracranially with the specified dilution( $10^{-1}$ ~ $10^{-5}$ ). For PMCAb, CWD seed was prepared as 10% homogenate diluted to a final dilution  $10^{-3}$  in 5%(w/v) normal TgElk mouse brain homogenate(NBH), with 3:7 dilution in NBH to a final dilution of  $1:10^8$ . Sonication was performed with Misonix 4000 sonicator with amplitude level 70, generating an average output of 160W with two teflon beads during each cycle. One round consisted of 56 cycles were set up in 96-well clear bottom optic plates and consisted of 98uL RT-QUIC buffer[final concentration of 1XPBS, 1mM EDTA, 10uM Thioflavin, 300mM NaCl buffer and 0.1mg/ml truncated(90-231, CSU-PRC) and full(23-231, impact) syrian hamster recombinant protein and 2uL of sample. The RT-QUIC assay was performed on a FLUOstar Omega fluorescence plate reader that was preheated to 42°C and 55°C for 60hr with 1min shaking at 700 rpm followed by 1min incubation.

**Results:** As the results summarized in Table 1 and the titer of the used CWD sample was 105.6 ic LD50/gram of elk brain. Using PMCAb, amplified PrP<sup>CWD</sup> was observed in  $10^{-3}$  and  $10^{-4}$  dilutions of CWD sample. In RT-QUIC assay, regardless of the length of the recombinant protein, positive conversions were detected up to  $10^{-6}$  at 42°C and up to  $10^{-7}$  at 55°C. The positive conversions were showed all reaction wells in  $10^{-3}$ ~ $10^{-5}$  dilution samples and only 2 of the 4 wells in  $10^{-6}$  and  $10^{-7}$  dilution samples.

**Conclusions:** We compared sensitivity of CWD prion detection methods including PMCAb, bioassay and RT-QUIC assay. The sensitivity of PMCAb and bioassay was consistent. RT-QUIC assay seems to be the most sensitive detection method among tested methods here. We need to test more normal brain samples to evaluated specificity of RT-QUIC assay.

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## **P51 Scrapie batch testing of TSE rapid tests in Italy: checking the consistency of performance**

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EU Member States carry out active surveillance on transmissible spongiform encephalopathies (TSEs) since 2001, according to Regulation (EC) No 999/2001. The aim is to monitor trends in disease evolution and prevalence. Tests approved in EU for the surveillance of TSEs in ruminants

are subject to a specific evaluation procedure. After being successfully evaluated, tests are listed in Annex X to Regulation (EC) N. 999/2001. EURL for TSE currently implements a batch testing procedure for BSE rapid test kits. The aim of such programme is to compare different batches of a particular test kit for consistency of performance. Indeed, a batch testing for scrapie test kits is not in place, although it is well known to insiders that there is not a clear consistency in the sensitivity of kit batches to atypical scrapie. Here we describe how the Italian Reference Laboratory for TSEs verifies the performance of scrapie batch test kits through an *ad hoc* procedure. The IDEXX HerdChek™ BSE- scrapie antigen test kit is applied in the net of the 12 Italian official rapid test laboratories. CEA batch testing activity includes a laboratory and a field step. A panel of 20 weak classical scrapie positive and 23 negative homogenates diluted according to EURL standard method of homogenate preparation (50% w/v protocol) are tested in double both with the current than the new batch. In the same test plate, a pool of atypical scrapie neat tissues diluted ½ in IDEXX buffer just before use is tested. The testing exercise is then performed as per the manufacturer's instructions. General quality standards for repeatability and randomization are observed. If the new batch passes the laboratory phase, it is checked in field conditions. Thus, routine samples are tested in parallel with the two batches for 3 consecutive days. Field positive samples are also tested during this step. In the last three years, 14 lots have been assessed through this procedure. The results obtained must fall within pre-determined ranges. If this occurs, it confirms that the new kit is essentially the same as previous batches and may be used with confidence. If the results are not within the ranges, it suggests that the kit has minor performances in comparison with the ones produced previously and is rejected. To date, 2 batches were rejected due to a lack of consistency in sensitivity on atypical samples. A European unified approach to eliminate duplication of effort has been discussed without reaching a conclusion, therefore, currently, a scrapie batch release testing and/or approval is carried out independently to varying degrees by European Member States. Indeed, it is well known how atypical strains can be challenging for diagnostic systems in use. On the basis of our experience a batch testing procedure aiming at checking continuous suitability of kit batches for classical and atypical scrapie ensures a good disease epidemiological monitoring.

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## **P52 EU-approved rapid tests detect L-type bovine spongiform encephalopathy in goat**

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The animal Transmissible spongiform encephalopathies were not regarded as zoonotic until the emergence of variant Creutzfeldt Jakob disease, connected to bovine spongiform encephalopathy (BSE), now referred as classical BSE (C-BSE). There is no data of naturally occurring C-BSE in sheep, whereas two cases were confirmed in goats, one in France and one in UK. Since 2004 two types of BSE with different phenotypical characteristics, commonly referred as "atypical" have been detected. These two atypical types, named high-type (H-BSE) and low-type (L-BSE) or bovine amyloidotic spongiform encephalopathy (BASE), are characterized by distinct Western Blot profiles of disease-associated prion protein. Atypical L-BSE is experimentally transmissible to sheep and these animals were likely exposed to BSE-contaminated feed in the early 1980s. There is concern that the atypical BSE agent may circulate in small ruminant population, representing a possible secondary risk to humans. No cases of natural occurring atypical BSE in sheep and goat were ever reported. Previous studies showed how BSE EU approved rapid tests detect atypical BSE in bovines despite evidence of differences in relative analytical sensitivity,

while some concerns emerged on the systematic sensitivity of rapid tests on the diagnosis of C-BSE in goats. Indeed, the laboratory performance of rapid diagnostic systems in the detection atypical BSE strains in small ruminants has not been studied to date. Here we report on the diagnostic sensitivity of EU-approved rapid tests for the detection of L-BSE in experimentally infected goats.

C-BSE and L-BSE isolates from bovine species were intracerebrally transmitted to goats, with a 100% attack rate (Italian Ministry of Health n. 694/2015-PR of 17<sup>th</sup> of July 2015). In order to compare the diagnostic sensitivity of EU approved rapid tests, *medulla oblongata* samples from 4 C-BSE, 5 L-BSE infected goats and 2 samples from control animals were tested in parallel with IDEXX HerdChek™ BSE- scrapie EIA Short and Ultra Short protocol - ovine conjugate, IDEXX HerdChek™ BSE- scrapie EIA Short and Ultra Short protocol - bovine conjugate; Bio-Rad TeSeE™ and Bio-Rad Sheep and Goat™. The assays were carried out according to the manufacturers' instructions. General quality standards for repeatability were observed. All samples from TSE-positive and negative animals were confirmed by Western blot or immunohistochemical analyses as required by EU Regulation (EC) N. 999/2001.

All tests and protocols were able to detect both C-BSE and L-BSE showing Bio-Rad tests some quantitative reduction in optical density in comparison to the IDEXX tests both for C-BSE than L-BSE samples. These data endorse the current epidemiological follow up and interpretation of such BSE forms, should they arise in goats. This means that the active surveillance applied to fallen stock and/or healthy slaughtered populations would detect such cases, reassuring on the epidemiological conclusions.

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### **P53 Effect of K222 PRNP Polymorphic Variants in the Susceptibility of Goats to intracerebral inoculation with caprine and bovine BSE**

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Prion diseases are fatal neurodegenerative diseases known to affect many mammalian species, including humans. TSEs in animals include scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease (CWD) in cervids. Scrapie is not regarded as a human health risk, whereas BSE represented one of the largest food crises in the last decades. EU efforts against BSE managed to almost eradicate the disease in the bovine population. However, the possibility that sheep and goats were also exposed to the same feedstuffs lead to several experimental studies, being both species successfully infected by intracerebral and oral route, and even naturally in an experimental flock. In 2005 it was confirmed the first case of truly natural BSE in a small ruminant: a goat from France, and one year after, a retrospective study found out another case in a goat from UK. Recent studies have suggested that K222 allele confers high resistance against scrapie and caprine BSE after oral challenge. This study compare two experimental studies that attempt to define the importance of different mutations at codon 222 of PRNP gene in the distribution of PrP<sup>Sc</sup> in goats after intracerebral inoculation with BSE. In the first study, nine animals were inoculated with caprine BSE agent; while in the second study, five animals were inoculated with bovine BSE. In both studies, goats carried the homozygous genotypes Lysine 222K

K and Glutamine 222QQ; and in the first study also the heterozygous Glutamine/Lysine 222QK. Most of 222QQ animals from both studies presented clinical signs after 15 months post inoculation in case of caprine BSE goats, and after 19±2 months in bovine BSE individuals. The 222KK animals inoculated with caprine BSE presented clinical signs 22 months after inoculation and the bovine BSE goats after more than 29 months, which indicate that the presence of K222 allele only increase the incubation period of the disease but not confers total resistance in goats inoculated with BSE. The distribution of PrP<sup>Sc</sup> in the CNS has been evaluate by immunohistochemistry, and the phenotype of PrP<sup>Sc</sup> accumulation was very similar in both groups of animals, existing only differences concerning the magnitude of deposition, which varied depending on the genotype and inoculum. In addition, a wide distribution of PrP<sup>Sc</sup> was observed throughout the organism, but always associated with lymphoid tissue or peripheral nerve fibers. It has been observed that in goats, allele K222 of the PRNP gene extends the incubation period of caprine BSE after intracerebral challenge, but does not protect totally against it. Apart from that, the longer incubation period in the 222KK animals inoculated with caprine BSE allows centripetal dissemination of PrP<sup>Sc</sup> to the tissues farthest from the point of inoculation, which may represent a risk for the maintenance of security in the food chain.

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#### **P54    Authentic prion propagation in PrP transgenic *Drosophila***

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Mammalian prions cause fatal neurodegenerative diseases such as Creutzfeldt-Jakob Disease (CJD) in humans, Bovine Spongiform Encephalopathy (BSE) in cattle and scrapie of sheep. The only reliable method to detect prion infectivity is by bioassay in a suitable experimental host, typically mice. The mouse bioassay is cumbersome, time consuming and financially very expensive. As such, there is an important need for development of a new prion bioassay, one that is relatively rapid, as equally sensitive as the mouse prion bioassay and financially economic. We have tested the hypothesis that authentic prion propagation occurs in PrP transgenic *Drosophila* in order to validate this invertebrate host as a new bioassay for mammalian prions.

We have shown that mammalian prions faithfully propagate in a strain-specific manner in PrP transgenic *Drosophila*. Adult ovine PrP transgenic *Drosophila*, exposed to mammalian prions at the larval stage, showed the progressive accumulation of infectious prions that were transmissible to mice and the presence of Proteinase K-resistant PrP, two hallmark features of bona fide mammalian prions. Strikingly, the biological properties of the original prion strain used to inoculate ovine PrP transgenic *Drosophila* were maintained during their passage in the fly.

Our data show that the molecular and cellular co-factors necessary for strain-specific prion propagation are not unique to mammalian species but conserved across millennia, and establish *Drosophila* as a novel host for the bioassay of transmissible mammalian prions.

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#### **55    Soil amendment with CAC-717 solution also acts as a prion disinfectant**

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We obtained a new electrically charged material (CAC-717) by continuously applying an electric field to mineral water containing calcium hydrogen carbonate. A Teflon insulation-coated electrostatic field electrode (N-800N, Mineral Activation Technical Research Center, Tamana, Japan; Japan Patent No. 5864010) was used to create the electric field at a voltage of  $2 \times 10^4$  V for 48 h. CAC-717 solution in distilled water (Japan Patent No. 5778328, FDA/USA Regulation No. 880.6890 Class 1 disinfectant) had a pH of  $12.39 \pm 0.03$  and contained calcium hydrogen carbonate particles (1,120 mg/L) and carbon complex microparticles (50–500 nm) with a mesoscopic structure.

CAC-717 solution was subsequently sprayed onto normal soil then growth of different crops observed for 6 months. Yield, as calculated by leaf weight, was then determined. The weights of cabbage, green tea, and kale increased 1.5–2.0-fold compared with plants grown on soil without CAC-717 solution. These findings suggest that CAC-717 also acts as an effective disinfectant against *Alternaria porri* (black-spotted disease in cabbage and kale).

Hamster brain prion sample (263 K) homogenates were subsequently mixed with nine times the volume of CAC-717. As a negative control, normal brain homogenates or recombinant normal cellular prion protein was diluted 10-fold with distilled water. Prion samples mixed with nine times the volume of distilled water were used as a second negative control. Mixtures were incubated at room temperature for 1 h then immediately diluted in ELISA buffer before determining the amount of prion in each sample using Western blotting with 3F4 monoclonal antibody (Prionics). A marked decrease in prion was observed in the samples mixed with CAC-717 solution.

Soil structure, nutrient content, composition, and microbiological activity tend to increase following organic amendment. Numerous studies have therefore examined the effect of organic soil amendment on crop yield and quality, soil erosion and nutrient availability, and bioremediation of heavy metals. Our future aim is to determine the microbiological activity of soil after spraying with CAC-717.

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## **P56 Prion-induced changes in *Drosophila* NMJ synaptic architecture**

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Prion diseases are fatal transmissible neurodegenerative diseases of humans and various other vertebrate species. Within an ongoing prion disease, synaptic loss or dysfunction precedes the onset of clinical signs. Central to prion disease is the conversion of the normal host protein PrP<sup>C</sup> into the abnormal conformer PrP<sup>Sc</sup>. The disease-associated form of PrP is considered to be responsible for prion-induced neurotoxicity. Mature PrP<sup>C</sup> is attached to the plasma membrane by a glycosylphosphatidyl-inositol anchor, while during its biosynthesis and metabolism, cytosolic, and secreted forms of the protein may arise. It is important to clarify the role of topological variants of PrP<sup>C</sup> in prion-induced neurotoxicity in order to understand the mechanism of neurodegeneration in prion diseases. This area of prion biology is difficult to study in natural hosts of these conditions because of the long incubation times for the development of clinical disease.

*Drosophila melanogaster* has emerged as a model system for the study of mammalian neurodegenerative disease. In particular, the neuromuscular junction (NMJ) of motor neurons innervating body muscles in *Drosophila* larvae have been used to study synaptic structure and function [1]. We have previously shown that transmissible mammalian prion disease can be modelled in PrP transgenic *Drosophila*. Our group has reported that adult ovine PrP transgenic *Drosophila*, exposed to ovine prions at the larval stage, develop a transmissible toxicity that is characterised by the accumulation of disease-associated PrP [2]. Here we used the *Drosophila* larvae NMJ model to probe the role of PrP directed to the membrane [PrP(GPI)], to the cytosol [PrP(Cyt)], or for secretion [PrP(deltaGPI)] in prion-induced changes in synaptic architecture.

We have found that while ovine PrP(GPI) had no effect on the overall morphology of the *Drosophila* larval NMJ, more diversity was induced by PrP(Cyt) and PrP(deltaGPI) expression, with or without exposure to ovine prions. As a consequence of prion-exposure, larval NMJ single bouton area was decreased in prion-exposed PrP(GPI) and PrP(cyt) *Drosophila*, which was not seen in those transgenic for PrP(deltaGPI). In addition, the total bouton count in prion-exposed VRQ(cyt) larval NMJs was increased, which was not evident in PrP(GPI) or PrP(deltaGPI) transgenic *Drosophila*. These data show that subcellular changes are evident in the synaptic architecture of prion-exposed larval NMJ of PrP transgenic *Drosophila* and that different topological variants of PrP<sup>C</sup> can participate in those processes that lead to these phenotypic changes.

#### Literature:

1. Menon, K.P., R.A. Carrillo, and K. Zinn, *Development and plasticity of the Drosophila larval neuromuscular junction*. Wiley Interdiscip Rev Dev Biol, 2013. **2**(5): p. 647-70.
2. Thackray, A.M., et al., *Prion-induced and spontaneous formation of transmissible toxicity in PrP transgenic Drosophila*. Biochem J, 2014. **463**(1): p. 31-40.

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#### **P57 A spontaneous misfolding-associated polymorphism in ovine *PRNP*(M112I) renders ShTg mice highly susceptible to atypical scrapie**

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Spontaneous misfolding of human PrP is a long-known event leading among other disorders to sporadic CJD, the most prevalent prion disease in human beings. However, little is known about pathogenesis of sporadic prion diseases in other mammals such as sheep. The high cost associated to ruminant housing and the scarcity of sporadic TSE cases makes necessary to develop reliable mouse models of these diseases not only to study the pathogenesis but also to test therapeutic approaches.

*In vitro* amplification experiments using recombinant PrP from different species as substrates show that certain amino acid changes render the PrP<sup>C</sup> highly susceptible to spontaneous misfolding in unseeded amplification reactions. These changes, when overexpressed in transgenic mice, cause a spontaneous and transmissible prion disease.

Certain, naturally occurring polymorphisms were identified in the equivalent positions in the *PRNP* of sheep. In particular, polymorphism M112I in the ovine *PRNP* gene showed enhanced spontaneous misfolding susceptibility *in vitro*. Thus, as previously done for bank vole and mouse, our objective is to determine if a transgenic mouse model over-expressing this polymorphic PrP<sup>C</sup> will give rise to a spontaneous and transmissible ovine prion disease. Brains of preclinical and clinical transgenic mice will be used to produce inocula that will be tested for TSE infectivity in known ovine and human transgenic mouse models.

Subsequently, several mouse lines have been generated, on a mouse-*PRNP* knock out background, with different sheep (ARQ) M112I PrP<sup>C</sup> expression levels. Two candidate lines have been selected with expression levels of 1.5x and 3x of the PrP<sup>C</sup> transgene (compared to sheep) in homozygosity.

Since in other transgenic models with this substitution the generated spontaneous prions were atypical-like, we challenged our model with ovine classical and atypical prions to determine its susceptibility. Even though no conclusive indication of spontaneous prion disease has been observed so far in the two lines under study, inoculation with atypical scrapie produced conspicuous neurological clinical signs, brain spongiosis and PrP<sup>res</sup> deposits as early as 140 days post inoculation (dpi), with a 100% attack rate (mean incubation period of 225 dpi) while animals inoculated with classical scrapie remain free of disease at >340 dpi. Indicating that the M112I substitution is highly permissive to atypical ovine prion misfolding.

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## **P58 RT-QuIC Seeding Activity Using Elk Recombinant Prion Proteins Discriminates Scrapie Isolates**

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Aims: Prions are amyloid-forming proteins that cause transmissible spongiform encephalopathies through a process involving conversion from the normal cellular prion protein (PrP<sup>C</sup>) to the pathogenic misfolded conformation (PrP<sup>Sc</sup>). This conversion has been used for *in vitro* assays including serial protein misfolding amplification and real-time quaking induced conversion (RT-QuIC). RT-QuIC has been used for the detection of prions in a variety of biological tissues from humans and animals. Extensive work has been done to demonstrate that RT-QuIC is a rapid, specific, and highly sensitive prion detection assay. RT-QuIC uses recombinant prion protein to detect minute amounts of PrP<sup>Sc</sup>. RT-QuIC has been successfully used for the detection of prions and for strain discrimination in a variety of biological tissues from humans and animals. However, RT-QuIC has not been shown to be useful to detect or discriminate different genotypes of sheep scrapie with recombinant elk prion proteins.

Methods: To do this, we used the RT-QuIC assay to measure of prion seeding activity. Elk recombinant prion protein was seeded with brain tissue samples from different strains and different genotypes of sheep infected with the scrapie agent. We also inoculated transgenic mice expressing M132 elk *PRNP* (Tg12) with scrapie from different genotypes of sheep to compare with our RT-QuIC results.

Results: We found that reaction mixtures seeded with genotype VRQ/VRQ sheep brains have better conversion efficiency with elk substrate M132 compared to reactions seeded with the



genotype ARQ/ARQ sheep brains regardless of strain types used. Our bioassay data also supported RT-QuIC results showing significantly shorter incubation period for inoculum from VRQ/VRQ sheep when compared to inoculum from ARQ/ARQ sheep.

Conclusions: Therefore, we conclude that *PRNP* background of both donor and recipient can strongly influence susceptibility, so pooled inocula historically used in prion transmission studies may make drawing concrete conclusions regarding strains difficult.

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**P59 No evidence of a negative effect on goat milk production traits associated with K222 polymorphism. A cross-sectional study in Chamois Coloured herds**

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Introduction. In goats several studies reported that the K222 polymorphism of the PRNP gene is associated with a lower risk of developing classical scrapie. Despite its low frequency and heterogeneous distribution in goat breeds, K222 is one of the main targets of breeding programs to contrast caprine scrapie. The aim of this study was to assess whether the K222 polymorphism has a negative impact on production traits in goat herds as studies on this issue are missing.

Materials and Methods. We enrolled two dairy herds of Chamois Coloured goats, where part of the animals carried K222. In both herds, a certain number of animals were selected based on their genotype and including both resistant and susceptible individuals. Milk yield was recorded and milk composition was analyzed periodically: protein, fat and lactose as well as somatic cell count (SCC) were determined. In total 180 milk samples from 93 animals (43 of them carrying the K222 polymorphism) were collected between January 2017 and February 2018. Hierarchical regression models were fitted using as dependent variables each of the above parameters, genotype and age class as explanatory variables, and taking into account random effects from repeated measures and herds.

Results. In the two herds the average milk yield per animal was similar (1.2 and 1.6 kg respectively) as were other traits (i.e. fat % 4.35 vs. 4.11; protein % 4.19 vs. 3.57; lactose % 4.19 vs. 4.41; median SCC 2,845 vs. 945 \*1000/ml). None of the four parameters considered were influenced by genotype ( $p>0.05$ ), although an age effect was observed, with decreasing protein levels in older animals.

Conclusion. In this preliminary small size study of Chamois Coloured goats, K222 polymorphism did not show any negative influence on production traits that would discourage the implementation of breeding programs to increase it.

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**P60 Cytokine expression in the progress of scrapie**

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### Introduction

It is currently assumed that neuroinflammatory mechanisms have a role in the neurodegenerative process associated with prion diseases. As a result, an immune response mediated by cytokines is believed to be involved in this inflammatory process. In fact, the presence of these signalling proteins has been assessed in several human neurodegenerative diseases, demonstrating that they are expressed by different cellular types. Moreover, an altered profile of inflammatory intermediaries during prion disease has been evidenced in some experimental murine models. Nevertheless, differences in relation to the induction of these proteins through the progress of neurodegeneration in natural model have not been studied to date. The objective here is to assess some of these inflammatory mediators in ovine Scrapie at different clinical stages.

### Methods

Immunohistochemical (IHC) protocols by using specific primary antibodies against several pro- and anti-inflammatory cytokines were applied in cerebellum tissue from 23 Scrapie affected sheep at preclinical (7), clinical (8) and terminal (8) stages. In addition, 8 healthy animals were used as control. A semi-quantitative score (from negative to maximum, +++) is performed in this study corresponding to each sample analysed after assessment by light microscopy. Immunostaining intensity was scored in each cerebellar layer (molecular, Purkinje cell and granular layers) as well as white matter.

### Results

IHC showed evident changes in the expression of cytokines assessed in Scrapie affected sheep compared to healthy controls. Despite most of them were distributed throughout all cerebellum layers, some remarkable findings consisted of the highest concentration associated with Purkinje cell layer for specific cytokines while it was observed in white matter for others.

### Conclusions

The increase of some markers related to inflammation in sheep cerebella belonging to different clinical stages of natural ovine Scrapie has been demonstrated in this preliminary study. By means of identification of cellular type expressing these inflammatory proteins, paying particular attention to astroglial and microglial populations, is expected to provide a global profile of the neuroinflammatory process in prion diseases.

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## **P61 Early Detection of Pathological Prion Protein in Cerebrospinal Fluid of Goats at the Asymptomatic Stage using Real-Time Quaking-Induced Conversion Assay**

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The spread of Bovine Spongiform Encephalopathies (BSE) agent to small ruminants is a major issue in the surveillance of Transmissible Spongiform Encephalopathies (TSEs) because BSE passage into a new host may change strain properties and make it difficult to recognize the original strain, also increasing the risk of epidemic spread. Since 2005, two natural BSE cases have been reported in goats. Furthermore, experimental transmissions of classical (C-BSE) and atypical form of BSE (L-BSE) in goats were also reported. Currently, small ruminant TSEs are definitively

diagnosed by *post-mortem* examination of brainstem. A top priority to minimize further spreading of prion diseases in small ruminants is the development of a highly sensitive, and specific test for *ante-mortem* detection of infected animals. Recent works reported high diagnostic value of second generation of cerebrospinal fluid (CSF) Real-Time Quaking-Induced Conversion (RT-QuIC) assays across the wide spectrum of human prions.

Here, we applied improved QuIC (IQ-CSF) for highly efficient detection of TSEs prion protein in goat cerebrospinal fluid. IQ-CSF sensitivity and specificity were evaluated on CSF samples collected at endpoint from goats naturally and experimentally infected with Scrapie or bovine isolates of C-BSE and L-BSE, respectively. Our findings indicated that we can sensitively detect pathological prion protein (PrP<sup>Sc</sup>) associated seeding activity using IQ-CSF in less than 24 hours. Next, CSF samples collected from L-BSE infected goats during pre-symptomatic stage were also analyzed. PrP<sup>L-BSE</sup> associated seeding activity was detected at early time points after experimental inoculation, with an average time of 439 days before clinical symptoms appeared. Finally, endpoint dilution analyses of CSF samples collected at clinical onset and about one year before, revealed that PrP<sup>Sc</sup> associated seeding activity was comparable.

Taken together these data are indicative of the great potential of this *in vitro* prion amplification assay as *ante-mortem* TSE test for live and asymptomatic small ruminants.

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## **P62 PrP<sup>Sc</sup> formation and clearance as determinants of prion tropism**

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Prion strains are operationally defined by their strain-specific neuropathology, incubation period, clinical signs, host range, and tissue tropism. The hyper (HY) and drowsy (DY) strains of hamster-adapted transmissible mink encephalopathy have distinct tissue tropism and susceptibility to disease via various routes of inoculation. Particularly, pathogenesis of the DY strain is restricted when inoculated via extraneural routes of infection. In contrast to HY, DY is not detected in secondary lymphoreticular system (LRS) tissues of infected hosts regardless of the route of inoculation.

In this study we found that HY and DY strains, are able to cross mucosal epithelia, enter the draining lymphatic vessels in underlying laminae propriae, and are transported to several LRS tissues. Since DY is able to cause disease once it enters the peripheral nervous system, the restriction in its pathogenesis is not due to a failure of transport but may be due to its inability to establish infection in LRS tissues.

To determine if DY can propagate in LRS tissues, we performed protein misfolding cyclic amplification using spleen homogenate as the source of PrP<sup>C</sup>. Our results show that the spleen environment can support DY PrP<sup>Sc</sup> formation, albeit at lower levels compared to lymphotropic prion strains, suggesting that the restriction in DY pathogenesis is not due to the lack of a strain-specific cofactor in spleen. Additionally, we found that DY PrP<sup>Sc</sup> is more susceptible to degradation compared to PrP<sup>Sc</sup> from other lymphotropic strains. Based on these results, we suggest that the balance between PrP<sup>Sc</sup> formation and clearance plays a crucial role in DY tissue tropism.

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**P63    MicroRNA alterations in a mouse model of prion disease**

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MicroRNAs (miRNAs) contribute to the development and pathology of many neurodegenerative diseases. Although our knowledge on the role of miRNAs in prion diseases has advanced recently, we are far from understanding their contribution to these pathologies. Besides their role in molecular pathology, small RNAs may provide also candidates for diagnostic markers because they are stable in body fluids where they enter by active processes (such as vesicular secretion) or passively from damaged cells and tissues. In order to investigate miRNA alterations in a mouse model of prion disease, Tg501 mice expressing the wt goat PRNP were intracranially inoculated with caprine scrapie strain. MicroRNAs from cervical spinal cord of presymptomatic and symptomatic animals and age-matched controls were quantified by small RNA sequencing. After multiple correction, only one and six significant miRNA alterations were found in presymptomatic and symptomatic animals, respectively. The results in their current state suggest that majority of the miRNA alterations are associated with the advanced pathology, which may limit their use for diagnostic purposes. The analysis of these miRNAs in different parts of the CNS and blood plasma of Tg501 model will be presented.

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**P64    A comparison of conventional and amplification prion detection methodologies with animal bioassay**

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Bioassay remains the gold standard for the detection of prion infectivity, and has provided tremendous insight to our understanding of prion pathogenesis and transmission dynamics. The conventional prion detection methods, IHC and western blot, have been heavily relied upon for their ability to detect PrPSc deposition in tissues of prion-infected hosts. In fact, IHC is considered the gold standard diagnostic test to demonstrate prion-infection. The recent introduction of assays employing amplification of prion seeds present in tissues and bodily fluids of infected hosts, sPMCA and RT-QuIC, have enhanced our ability to identify prions during the earlier phases of disease and in samples containing minute quantities of prions. This new generation of amplification assays have reported sensitivity levels rivaling that of bioassay. Thus, a variety of methodologies have been used to detect prions, yet to date, a cross platform comparison of these assays has not been performed.

We compared conventional (IHC) and amplification assays (sPMCA and RT-QuIC) to determine their ability to detect prions in brain tissue harvested from a CWD mouse dilutional bioassay. We demonstrate that conventional IHC performs well in the detection of PrPSc deposition across the

dilutional series. We also reveal enhanced detection of prion seeding activity in brain tissue at the lower end of the dilutional series if RT-QuIC is used as the readout for sPMCA.

In summary, we provide evidence that the new generation of amplification assays enhance prion detection sensitivity and rigor over conventional assays. While in vitro detection methodologies will never replace bioassay, they may be used as tools to augment detection, alleviate the time and cost (financial and animal life) of animal bioassay, and become tools for the early diagnosis of human and animal protein misfolding disorders.

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#### **P65 Tetraspanin 15 differentially regulates shedding of various ADAM10 substrates and impacts on prion disease in mice**

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The metalloprotease ADAM10 is the principal sheddase responsible for the enzymatic release of the prion protein (PrP) from the plasma membrane. Recent studies revealed that this shedding of PrP significantly impacts on prion diseases and may influence other pathological conditions of the brain as well. Despite the multitude of ADAM10 substrates in different cell types/organs and its obvious therapeutic potential in various diseases, knowledge on how this protease is regulated is surprisingly limited. Some members of the tetraspanin family have been suggested to specifically associate with ADAM10 and to differentially regulate its activity towards certain substrates in different tissues. Based on previous findings showing specific interaction between tetraspanin 15 (Tspan15) and ADAM10, Tspan15 knockout mice were generated and characterized in detail. Although these mice did not present with an obvious phenotype, a decreased expression of mature ADAM10 was found in the brain. This decrease correlated with reduced cleavage of some (e.g. N-cadherin and, most notably, PrP) yet not all prominent ADAM10 substrates (e.g. APP, Notch), thus supporting the hypothesis of a differential influence of certain tetraspanins on the cleavage of specific ADAM10 substrates. Importantly, lack of Tspan15 and reduced shedding of PrP resulted in significantly shortened incubation times in prion-inoculated mice. Among other findings, this study demonstrates relevant effects of a tetraspanin member on prion protein biology and prion diseases.

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#### **P66 Independent amplification of co-infected long incubation period slowly replicating prion strains**

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Prion diseases are caused by a misfolded isoform of the prion protein, PrP<sup>Sc</sup>. Prion strains are hypothesized to be encoded by strain-specific conformations of PrP<sup>Sc</sup>. Prion strains can interfere with each other when a long incubation period strain (i.e. blocking strain) inhibits the replication of a short incubation period strain (i.e. non-blocking).

Prion strain interference influences prion strain dynamics and the emergence of a strain from a mixture, however, it is unknown if two long incubation period strains can interfere with each other. Co-infection of animals with combinations of long incubation period strains failed to identify evidence of strain interference. To exclude the possibility that this inability of strains to interfere *in vivo* was due to a failure to infect common populations of neurons we used protein misfolding strain interference (PMCA<sub>si</sub>). Consistent with the animal bioassay studies, using PMCA<sub>si</sub> we found evidence that both co-infecting strains were amplifying independently, suggesting that the lack of strain interference is not due to a failure to target the same cells but is an inherent property of the strains involved. Additionally, we found relatively higher levels of remaining PrP<sup>C</sup> remaining following PMCA reactions seeded with long incubation period strains compared to short incubation period strains and hypothesize that the failure of these slowly replicating prion strains to interfere with each other *in vivo* or *in vitro* is that PrP<sup>C</sup> is not rate limiting.

Overall, this observation changes the paradigm of the interactions of prion strains in a mixture and has implications for interspecies transmission and emergence of prion strains from a mixture.

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#### **P67 The prion replication-related proteins BAMBI and CHGA are upregulated in natural and experimental scrapie models**

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Genome-wide studies have provided evidence for the existence of candidate genes that could explain differences in susceptibility and incubation period in prion diseases. A transcriptomic study using N2a cells revealed a regulatory network associated with prion replication *in vitro*, including genes with a known role in extracellular matrix remodelling. Here we evaluate whether the genes described as potentially involved in the replication of the prion protein could also play a role in scrapie pathology *in vivo*. For this purpose, we performed a gene expression analysis in medulla oblongata of sheep naturally infected with scrapie in a clinical stage of ten of those potential biomarkers (*BAMBI*, *CHGA*, *DLC1*, *FNI*, *GALT*, *IL11RA*, *ITGA8*, *LRRN4*, *PAPSS2* and *RGS4*), which were shown to be downregulated in N2A clones susceptible to prion infection. Only *CHGA* (*Chromogranin A*) displayed a statistically significant negative regulation in scrapie medullae. In contrast, *BAMBI* (BMP and Activin Membrane Bound Inhibitor) was upregulated. *Bambi* and *Chga* gene expression was quantified in mesencephalon of mice overexpressing the ovine prion protein (Tg338 model), showing a downregulation of both genes in clinical scrapie. The distribution of proteins encoded by these two genes was evaluated by immunohistochemistry in the central nervous system of scrapie-affected sheep and Tg338 mice. In accordance with gene expression results, BAMBI immunostaining was moderately higher in scrapie sheep being significantly increased in the hypothalamus and the hypoglossal nucleus in medulla oblongata. Protein scores were also significantly higher in hippocampus, thalamus, hypothalamus, deep cerebellar nuclei and medulla oblongata of clinical scrapie Tg338 compared to controls. Surprisingly, CHGA staining was also stronger in scrapie sheep brains, being significantly higher in frontal cortex, medulla oblongata, and cerebellum. Similarly, this protein was significantly upregulated in thalamus, Purkinje cell layer and cerebellar white matter of Tg338 clinical mice. Although downregulation of these genes at the transcript levels was associated with prion replication *in vitro*, *BAMBI* transcript levels positively correlated with prion deposition in scrapie

animals. Moreover, the scores of BAMBI and CHGA immunostaining positively correlated with scrapie related lesions both within the total number of animals and in scrapie-affected animals alone. Our study suggests that *BAMBI* and *CHGA* could play a role in the neuropathology of prion diseases *in vivo* but their pathogenic mechanism seems to be different than the one described *in vitro*. Although the role of BAMBI in neurodegenerative diseases has not yet been investigated in depth, it could be related with neuroinflammation in Alzheimer's disease (AD). Similarly, CHGA is a proinflammatory inducer of neuroinflammation in AD. The possible relationship between these proteins and neuroinflammation in scrapie is currently under investigation.

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#### **P68    Transmission of Chronic Wasting Disease (CWD) into Syrian Golden Hamsters selects novel CWD strains**

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Chronic wasting disease (CWD) is currently increasing in prevalence and expanding its geographic range in wild and farmed cervids in North America and Europe. Given that this disease affects multiple cervid species with distinct polymorphisms, the diversification and emergence of prion strains seems likely. To this end, we are characterizing hunter-harvested CWD isolates and have identified novel CWD strains based on their biochemical properties and host range. Eleven CWD-positive hunter-harvested isolates (five from mule deer and six from white-tailed deer) were used to inoculate Syrian Golden hamsters and cervidized (Tg33; G96) mice. While all eleven isolates resulted in clinical infection of cervidized mice, transmission into Syrian Golden hamsters varied; four of the eleven isolates were negative for PrP-res at 2 years post-inoculation. Furthermore, two distinct PrP-res profiles were present, by western blot analysis, in brains of hamsters inoculated with one white-tailed deer isolate (WTD-02). One glycoform resembled other hamster-passaged white-tailed deer isolates, while the other resembled elk CWD passaged in hamsters. Interestingly, analysis of the deer brain homogenates by cervid cell assay (Elk21- cells), showed that WTD-02 had spot counts between those observed for white-tailed deer and elk, suggesting that WTD-02 may be a mix of white-tailed deer and elk prions. These data suggest host range variation in CWD isolates from wild type cervids, and demonstrate the utility of Syrian Golden hamsters in differentiating strains from cervids.

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#### **P69    Experimental transmission of CWD from white-tailed deer to co-housed reindeer**

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Chronic wasting disease (CWD) continues to be detected in wild and farmed cervid populations of North America, affecting predominantly white-tailed deer, mule deer and elk. Extensive herds of wild caribou exist in northern regions of Canada, although surveillance has not detected the presence of CWD in this population. Oral experimental transmission has demonstrated that reindeer, a species closely related to caribou, are susceptible to CWD. Recently, CWD was detected for the first time in Europe, in wild Norwegian reindeer, advancing the possibility that caribou in North America could also become infected. Given the potential overlap in habitat

between wild CWD-infected cervids and wild caribou herds in Canada, we sought to investigate the horizontal transmissibility of CWD from white-tailed deer to reindeer.

Two white-tailed deer were orally inoculated with a brain homogenate prepared from a farmed Canadian white-tailed deer previously diagnosed with CWD. Two reindeer, with no history of exposure to CWD, were housed in the same enclosure as the white-tailed deer, 3.5 months after the deer were orally inoculated. The white-tailed deer developed clinical signs consistent with CWD beginning at 15.2 and 21 months post-inoculation (mpi), and were euthanized at 18.7 and 23.1 mpi, respectively. Confirmatory testing by immunohistochemistry (IHC) and western blot demonstrated widespread aggregates of pathological prion protein (PrP<sup>CWD</sup>) in the central nervous system and lymphoid tissues of both inoculated white-tailed deer. Both reindeer were subjected to recto-anal mucosal associated lymphoid tissue (RAMALT) biopsy at 20 months post-exposure (mpe) to the white-tailed deer. The biopsy from one reindeer contained PrP<sup>CWD</sup> confirmed by IHC. This reindeer displayed only subtle clinical evidence of disease prior to a rapid decline in condition requiring euthanasia at 22.5 mpe. Analysis of tissues from this reindeer by IHC revealed widespread PrP<sup>CWD</sup> deposition, predominantly in central nervous system and lymphoreticular tissues. Western blot molecular profiles were similar between both orally inoculated white-tailed deer and the CWD positive reindeer. Despite sharing the same enclosure, the other reindeer was RAMALT negative at 20 mpe, and PrP<sup>CWD</sup> was not detected in brainstem and lymphoid tissues following necropsy at 35 mpe. Sequencing of the prion protein gene from both reindeer revealed differences at several codons, which may have influenced susceptibility to infection.

Natural transmission of CWD occurs relatively efficiently amongst cervids, supporting the expanding geographic distribution of disease and the potential for transmission to previously naïve populations. The efficient horizontal transmission of CWD from white-tailed deer to reindeer observed here highlights the potential for reindeer to become infected if exposed to other cervids or environments infected with CWD.

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#### **P70 Evaluation of miRNA levels and infectivity of exosomes from plasma and cerebrospinal fluid in ovine scrapie**

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Scrapie is a prion disease that affects sheep and goats. Scrapie diagnosis is currently based on the post-mortem detection of the abnormal form of the prion protein in brain tissues. Genomic tools can allow the identification of accessible, rapid and economical early biomarkers for diagnostic use. Some microRNAs (miRNAs) have been reported to display altered expression in the central nervous system (CNS) of mice infected with scrapie, in samples of human TSEs and in exosomes released by prion-infected cells. In addition, PrP<sup>c</sup> is part of the exosomal cargo. The infectivity of exosomal fraction in plasma from mice infected with a human TSE has been proved, but both this infectivity and epigenetic changes have never been studied in the natural form of the disease.

Here, miRNAs alterations were studied in cervical spinal cord (CSC) of naturally infected scrapie sheep by quantitative real time PCR. To obtain accessible biomarkers for TSE diagnosis, selected



miRNAs were analysed in circulating blood plasma, cerebrospinal fluid (CSF) and exosomes from these biological fluids. Plasma and CSF exosomes from clinical and preclinical scrapie sheep and control sheep were isolated using commercial kits and ultracentrifugation; characterized by western blot using antibodies against exosomal markers HSP70, CD63 and CD81 and non-exosomal markers such as mitochondrial Cytochrome C; and analysed for morphology by transmission electron microscopy (TEM). The infectivity of sheep exosomes was evaluated by protein misfolding cyclic amplification (PMCA) and by inoculation in Tg338 transgenic mice.

Only miR-21-5p displayed a close-to-significant increase in scrapie CSC. However, the levels of three others correlated with histopathological lesions. In circulating blood plasma, miR-21-5p and miR-342-3p displayed significant increase, and only miR-21-5p was significantly increased in plasma exosomes from scrapie sheep. In total CSF, miR-21-5p, miR-146a-5p, miR-128a-3p were increased in scrapie animals, whereas miR-342-3p showed close to significant increase. Only two of the studied miRNAs (miR-342-3p and miR-486-5p) were expressed in sufficiently high levels in CSF-derived exosomes but there were no differences between scrapie and control sheep.

Plasma-derived exosomes did not show detectable pathological protein, whereas PrP<sup>Sc</sup> in CSF exosomes was successfully amplified in most samples from clinical scrapie sheep and one preclinical animal. Murine assays are still ongoing.

This is the first description of circulating and exosomal miRNA alterations in TSE naturally affected animals. Although PrP<sup>Sc</sup> detection in exosomes would not be a reliable method for early diagnosis of the disease, the detection of the prion protein in CSF exosomes could indicate that these vesicles are involved in prion propagation throughout the organism, which would contribute to the better understanding of the TSEs pathogenesis.

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#### **P71 Characterization of the Cervid Prion Protein Polymorphism S138N and Its Distribution in Caribou Populations in Western Canada**

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Polymorphisms within the cervid prion protein (PrP) sequence may influence its physiological processing and conversion ability. This consequently affects prion propagation during infection, resulting in altered pathogenesis and prion properties of Chronic Wasting Disease (CWD). We investigated cervid PrP harboring a polymorphism at codon 138 from serine to asparagine (S138N) found in caribou that has been associated with prolonged incubation periods of CWD. How the S138N polymorphism produces this effect is yet to be studied. Hence, we analysed the proteolytic processing and in vitro conversion efficiency of the S138N PrP. We also determined the distribution of this polymorphism in caribou populations in Western Canada to assess the risk of CWD infection.

#### **Method:**

We transiently transfected neuronal and non-neuronal cell lines depleted of endogenous PrP (PrP<sup>C</sup>) to express cervid wt and 138N PrP. Cells used were CAD5 PrP knock-outs generated using the Crispr/Cas9 system and RK13 cells that do not produce endogenous PrP. Endogenous

processing of PrP, termed  $\alpha$ -cleavage, was determined using PNGase assay to remove N-linked glycans followed by quantification of PrP fragments by western blotting. We used real-time quaking-induced conversion (RT-QuIC) to test the ability of cervid wild-type and 138N recombinant PrP to form amyloid fibrils when seeded with CWD-positive cervid brain homogenate. We also sequenced the PrP gene in samples from several caribou populations in Western Canada and determined the 138N allele frequency.

#### Results:

We found subtle differences in the  $\alpha$ -cleavage processing of wild-type and 138N PrP that calls for further investigation. Also, we identified an additional PrP band that migrated slightly faster than the full-length PrP in the transfected RK13 cells. This band was not present in the typical PNGase assay results of our transfected CAD5 KOs. Our preliminary RT-QuIC results show that the 138N recombinant PrP seem to form amyloid fibrils as efficiently as the wild-type when seeded with CWD-positive elk brain homogenate. We determined that the 138N allele is present in caribou populations in Western Canada and an uncommonly high proportion of the allele (0.583) was found in the Chinchaga woodland caribou population.

#### Conclusion:

Our preliminary results show that other than  $\alpha$ -cleavage and conversion ability, there may be other endogenous processes and factors that is/are affected by this polymorphism. Our results also demonstrate that PrP processing may be different in different cell lines, therefore the choice of cell line in *in vitro* experiments is important. The presence of the 138N allele in caribou populations in Western Canada support the need to sequence more individuals from different areas, populations and species.

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### **P72 Effect of Route of Inoculation and Strain on PrP<sup>d</sup> Accumulation in Nodose and Superior Cervical Ganglia in Clinically-Ill Hamsters**

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The nodose ganglia (NG) contain visceral afferent neurons that innervate heart, lungs and gut and terminate centrally in the solitary nucleus. These cells are known to accumulate the disease associated prion protein (PrP<sup>d</sup>) when animals become clinically-ill following per os inoculation. The superior cervical ganglia (SCG) contain sympathetic postganglionic motor neurons that innervate structures in the head and neck, including blood vessels that supply the carotid body (CB). The first aim of this study was to examine accumulation of PrP<sup>d</sup> in NG of clinically-ill hamsters following intracerebral (i.c.) or intraperitoneal (i.p.) inoculation of HY or DY strains to determine if there was strain- or route- specificity to accumulation of PrP<sup>d</sup>. The second aim was to determine if PrP<sup>d</sup> accumulates in sympathetic neurons that are located in a structure that provides innervation to blood vessels that supply the CB.

Blocks of tissue surrounding the bifurcation of the common carotid artery were taken from uninfected (n=5), mock-infected (n=5) or clinically-ill male golden Syrian hamsters i.c.-inoculated with HY (n=10), or DY (n=5), or i.p. with HY (n=5). Tissue blocks containing SCG, NG, anterior cervical lymph nodes (LN) and CBs were embedded in paraffin for serial sectioning. Series of 3-4 adjacent tissue sections spaced not further than 100  $\mu$ m apart (3-4 per animal) were stained with hematoxylin and eosin, or immunohistochemically (IHC) processed for the presence of PrP<sup>d</sup> using the 3F4 antibody. Each IHC experiment included tissues from both control groups and each experimental group; comparisons were made between sections processed at the same time with the same solutions. Some tissue sections were IHC processed for tyrosine hydroxylase

(marker for catecholaminergic neurons) or synaptophysin (marker for synapses) to assist in differentiating SCG from NG.

SCG, NG, CB and LNs of uninoculated animals or mock-infected animals did not contain PrP<sup>d</sup>. PrP<sup>d</sup> was identified in SCG, NG, CB and LNs in all animals i.c.-inoculated with HY, but not in animals i.c.-inoculated with DY. PrP<sup>d</sup> was identified in CBs and NGs of all animals i.p.-inoculated with HY. PrP<sup>d</sup> was not identified in SCG cells and in LNs of just 2/5 animals i.p.-inoculated with HY. Amount and numbers of cells accumulating PrP<sup>d</sup> were consistently less in i.p.-inoculated versus i.c.-inoculated animals.

Accumulation of PrP<sup>d</sup> in NG cells of clinically ill-animals is strain-specific. PrP<sup>d</sup> fails to accumulate in clinically-ill animals inoculated with DY but does so robustly in HY-infected animals. Accumulation of PrP<sup>d</sup> in NG cells is not route-specific, since it can be detected in all i.c.- and i.p.-inoculated HY-infected animals. However, the amount of PrP<sup>d</sup> was much greater following i.c.-inoculation. Accumulation of PrP<sup>d</sup> in SCG cells was both strain- and route-specific, being detectable only in animals i.c.-inoculated with HY. These results do not support the SCG as an entry point for neuroinvasion.

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### **P73 Glycans are not necessary to maintain the pathobiological features of classical BSE**

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The implication of the glycosylation status of PrP<sup>C</sup> in the conversion to its pathological counterpart and on cross-species transmission of prion strains has been discussed at length. Here, we have evaluated the effect of the PrP<sup>C</sup> glycosylation on strain characteristics through the propagation of distinct BSE isolates over a human non-glycosylated PrP<sup>C</sup> mouse model. Bovine, ovine and porcine-passaged BSE, and vCJD isolates were used as seeds/inocula in both *in vitro* and *in vivo* propagation assays using the TgNN6h mouse model (Haldiman T *et al*, 2013). On PMCA, all isolates maintained the biochemical characteristic of BSE. On bioassay, except for vCJD, the direct inoculation of the original BSE isolates did not cause disease in a first passage. However, all PMCA-propagated prions were readily transmitted to TgNN6h mice, these results being in agreement with our previous *in vitro* data. Histological and Western-blotting analyses of brains from mice with neurological signs showed that the neuropathological and biochemical hallmarks of BSE were maintained after transmission to a humanized non-glycosylated host, suggesting that the absence of glycans did not alter its pathobiological features.

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### **P74 High Prevalence of CWD prions in male reproductive samples**

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Chronic wasting disease (CWD) is a highly infectious and fatal illness affecting captive and free-ranging cervids. Mother-to-offspring prion transmission has been described in some animal prion diseases, including CWD. However, few studies have been performed to analyze the prevalence of CWD prions in reproductive male tissues and fluids. Here, we optimized the Protein Misfolding Cyclic Amplification (PMCA) assay for the efficient detection of CWD prions in these samples. This study was done in collaboration with United States Department of Agriculture (USDA) scientists who provided blindly field-collected testes, epididymis and seminal fluid samples from 21 white-tailed deer that were analyzed for prion infection by *post-mortem* histological studies in brain stem and lymphoid tissues. The results showed positive CWD prion detection in testes, epididymis and seminal fluid samples. A high prevalence of CWD-PrP<sup>Sc</sup> was found in samples collected at the late-presymptomatic stage of the disease. Our results showed a correlation between the presence of CWD-PrP<sup>Sc</sup> in male reproductive organs and blood. These findings demonstrate a high efficiency of CWD prion detection by PMCA in testes, epididymis and seminal fluid, and offer a possibility for a routine screening of semen samples to be commercially distributed for artificial insemination. Our results may uncover new opportunities to understand the mechanisms of CWD spreading and decrease putative inter-individual transmission associated to insemination using CWD contaminated specimens.

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#### **P75 Determining transmissibility and proteome changes associated with abnormal bovine prionopathy**

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In prion diseases, it is believed that altered protein conformation encodes for different pathogenic strains. Currently 3 different strains of bovine spongiform encephalopathy (BSE) are confirmed. Diagnostic tests for BSE are able to identify animals infected with all 3 strains, however, several diagnostic laboratories have reported samples with inconclusive results which are challenging to classify. It was suggested that these may be novel strains of BSE; to determine transmissibility, brain material from index cases were inoculated into cattle.

In the first passage, cattle were intra-cranially challenged with brain homogenate from 2 Swiss animals with abnormal prionopathy. The challenged cattle incubated for 3 years and were euthanized with no clinical signs of neurologic disease. Animals were negative when tested on validated diagnostic tests but several research methods demonstrated changes in the prion conformation in these cattle, including density gradient centrifugation and immunohistochemistry. Currently, samples from the P1 animals are being tested for changes in protein levels using 2-D Fluorescence Difference Gel Electrophoresis (2D DIGE) and mass spectrometry. It is anticipated that, if a prionopathy is present, this approach should identify pathways and targets to decipher the source of altered protein conformation. In addition, a second set of cattle have been challenged with brain material from the first passage. Ideally, these cattle will be given a sufficient incubation period to provide a definitive answer to the question of transmissibility.

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**P76    Alteration of microglial activation by mesenchymal stem cells**

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Prion diseases are fatal neurodegenerative diseases in animals and human. Despite many efforts, effective treatments for prion diseases have not been established yet. Mesenchymal stem cells (MSCs) are multi-potent cells that can differentiate into cells of mesodermal origin such as osteocytes and adipocytes. Autologous transplantation of bone marrow-derived MSCs into stroke patients improved their clinical condition. Recently, we reported that autologous transplantation of mouse compact bone-derived MSCs (mCB-MSCs) into prion Chandler strain-infected mice prolonged the survival of mice. Interestingly, the mCB-MSCs transplantation enhanced further microglial activation with up-regulation of some of the marker genes for M2-type microglia. In order to elucidate the mechanism for the control of microglial activation with mCB-MSCs, we analyzed the influence of pathological niche of prion diseases on the activation of mCB-MSCs and the influence of factors produced from mCB-MSCs on microglial activation. Brain extracts from Chandler strain-infected mice stimulated mCB-MSCs with the up-regulation of *COX-2* gene expression, suggesting that mCB-MSCs can respond to niche of prion diseases in brain. When LPS-treated microglia were incubated with the conditioned medium from mCB-MSCs that were treated with brain extracts from Chandler strain-infected mice, the expression of *CD68*, one of the microglial activation markers, was down-regulated. Additionally, the expression of *IL-1 $\beta$* , a marker for M1-type microglia, was down-regulated, whereas the expression of *Arg-1*, a marker for M2-type microglia, was up-regulated. These results suggest that the mCB-MSCs treated with brain extracts from prion-infected mice have the ability to shift activation state of LPS-treated microglia from M1-type to M2-type. However, unexpectedly, conditioned mediums from mCB-MSCs treated with brain extracts from mock-infected mice or without brain extracts also appeared to have the same ability. Similar tendencies, down-regulation of *CD68* and *IL-1 $\beta$* , but up-regulation of *Arg-1*, were observed when microglia isolated from the Chandler strain-infected mice were used. These results suggest that, regardless of their activation state, mCB-MSCs intrinsically secrete factors that shift microglial activation state from M1 to M2-type. The finding in the current study suggests that factors secreted from MSCs as well as MSCs themselves will be applicable to the treatment of prion diseases and other neurodegenerative disorders.

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**P77    In vitro approach to estimate the human transmission risk of prions**

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Prion diseases are fatal neurodegenerative disorders in humans and animals. The key event in the pathogenesis of these disease is the conversion of host-encoded normal cellular prion protein (PrP<sup>C</sup>) into its pathogenic isoform (PrP<sup>Sc</sup>) and its accumulation in the central nervous system. One of the characteristics of prion is the species barrier that limits the transmission between

different species. Currently, bioassays using transgenic mice (Tg) overexpressing PrP of different species have become valuable tools for assessing cross species transmissibility of prions.

The recent reports describing the emergence of novel bovine spongiform encephalopathy (BSE) from H-BSE and the transmission of chronic wasting disease to swine have generated concerns of human infections of newly identified prions. Although Tg expressing human PrP have been used to model human susceptibility to animal prions, these experiments are costly and time-consuming. In addition, the results of bioassays are influenced by the lines of transgenic mice used and the lifespan of the challenged animals. These factors are needed to be taken into account when assessing the human risk of prions.

In attempt to develop the more time- and cost-saving method for assessment of the human transmission risk of prions, we performed experiments using protein misfolding cyclic amplification (PMCA) technique to investigate whether PMCA can be compatible with bioassay. Using brain homogenates of Tg expressing bovine PrP as the PrP substrate, we optimized the versatile PMCA condition that could amplify PrPSc from cattle affected with C-, H- or L-BSE. We measured the 50% PMCA seeding activity dose and the 50% lethal dose in 1 g equivalent of C-, H- or L-BSE cattle brain tissue by using PMCA or bioassay, respectively, and assessed the correlations between these doses.

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## **P78 Camino primitivo or BSE in the style of Pablo Picasso**

Becker A

Independent Institute for Holistic Prion Research.

My realizations taken from PRION 2017 Edinburgh:

1. Stanley Prusiner looked for a virus but only found a protein (infectious protein – inpro – proin – prion). That was like going to hell, there was no “Back to basics”, but Prusiner was further on going to hell, followed by the complete research community.
2. Up to now nobody understands the early event of infection, the seeding, taken part long before the occurrence of subclinical and clinical symptoms. Nobody knows in which brain region the disease starts and why animals without PrPc can live. The biological function of PrPc is still unknown. 80 percent of all scientific publications on prion diseases have been made in the last 5 years.
3. There was no answer to the question: Why BSE in Great Britain in the 1980's?

Since 2010 I am attending the international Prion conferences as a veterinary practitioner to draw scientist's attention to a holistic link between prions and Ivermectin intoxication. The only success was surprisingly winning the Poster Award at the meeting in Amsterdam.

For this reason, I decided – approximately 35 years after the outbreak of BSE – to walk 350 kilometers by foot to Santiago de Compostela using the “Camino primitivo”. There I will present a poster as a work of art, BSE in the style of Pablo Picasso, only containing three pictures according to the motto “A picture is worth a thousand words”.

Picture 1 shows how Stanley Prusiner transfers PrPSc to cattle by intracerebral injection. Considering PrPSc as one part of a “PrPc-PrPSc-complex”, a positively feed backed trigger mechanism, the transmission is not more than a triggering via chain reaction. Considering PrPSc only as an infectious particle, generating with all force a virus out of a protein because looking for a (slow) virus for decades, was the merit of Prusiner. But the reward of half the truth or half

the work with the award of the 1997 Nobel Prize for Medicine was a huge mistake leading research in a completely wrong direction.

Picture 2 stands for the introduction of the avermectins (Ivermectin) in 1982. Ivermectin is an endectocide (macrocyclic lactone), an antiparasitic drug that is active both against endoparasites and ectoparasites. Ivermectin extremely increases the susceptibility of bovines for BSE and can no longer be ignored as the most important co-factor. The award of the 2015 Nobel Prize for Medicine to the researchers Campbell (Merck US Pharma) and Omura for the discovery and development of avermectins must not cover up the dangerousness of those neurotoxic pesticides.

Picture 3 summarizes what happens if all conditions are met: The emergency of BSE in Great Britain, caused by reducing the temperature in rendering plants in the early 1980s, the marketing introduction of Ivermectin in 1982, the production of meat and bone meal without total extraction of fat, the use of animal fats in milk replacers, and the residues by antiparasitic treatment with Ivermectin especially during pregnancy.

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**P79 Clearance within dystrophic neurites accumulating autophagic vacuoles initiates neuritic destruction.**

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Background: Recently, findings suggesting the leakage of lysosomal enzymes from autolysosomes that initiate degeneration and, probably, the loss of neurites in neurodegenerations have been published. This prompted us to re-examine the samples of Echigo-1- or 263K-strain of scrapie-infected hamsters to look for the cytoplasmic clearance, not unlike of those other animal models.

Material and methods: For the experiment hamsters inoculated with Echigo-1 and 263K scrapie strains were used. Hamsters in the terminal stage of prion disease and control sham-inoculated hamsters at the same interval after inoculation were anaesthetized and perfused by intracardiac injection with glutaraldehyde and formaldehyde. The brain samples were routinely processed for electron and light microscopy. Grids were examined in Jeol 1100 transmission electron microscope.

Results: The ultrastructural picture of the Echigo-1 and 263K infected hamster brains consisted of spongiform change, astrogliosis, tubulovesicular structures and numerous dystrophic neurites. Dystrophic neurites were loaded with autophagic vacuoles of different stage and lysosomes. In both models in several neurites, we found electron-lucent areas not bound by any membranes or only partially bound; thus, they were not autophagic vacuoles as the latter are membrane-bound and contain cargo. In some neurites, these areas could be traced over several sections. Moreover, they were evident to change size, i.e. to expand.

Conclusions: It may be speculated that this ultrastructurally observed phenomenon is a result of autophagic vacuoles or lysosomal disruption leading to removal of axonal elements. Our results are in accordance with our earlier publications showing that impaired autophagy and lysosomal damage leads to the leakage of lysosomal enzymes in axons and evokes axonal damage.

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**P80 Amplification of prions from single cells and tissue sections isolated from the brain of infected animals by laser capture microdissection**

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A typical pathological feature of prion diseases is the massive accumulation of misfolded prion protein (PrP<sup>Sc</sup>) in the brain, in the form of protease-resistant aggregates. Depending on the prion strain, PrP<sup>Sc</sup> deposition may affect different areas of the brain and can appear attached to distinct nerve cells or extracellularly in the form of diffuse deposits and compacted amyloid plaques. Currently, the cellular factors and processes responsible for the specific tropism of different prions are not known. Also unknown are the strains, biochemical, biological and structural characteristics of PrP<sup>Sc</sup> associated to different nerve cells in an infected brain. Indeed, several previous reports have shown that different prion strains can co-exist in the same brain. In this study, we optimized the protein misfolding cyclic amplification (PMCA) technology to amplify PrP<sup>Sc</sup> from an individual cell. We also utilized the single-cell PMCA technique to analyze prions associated to different tissue sections and cells extracted from the brain of prion infected animals using laser capture microdissection (LCM). To study prion amplification from single cells, we used a cell line chronically infected with RML prions. Serial dilutions of an infected cell culture showed that PMCA enabled to amplify prions even from an estimated individual cell. To confirm this, we plated dilutions of cells and carefully examined under the microscope the exact amount of cells. Our data confirm that, indeed, PrP<sup>Sc</sup> can be serially replicated starting from a single infected cell. We also showed that PMCA can amplify tissue sections of different sizes extracted by LCM from distinct areas of the brain of mice infected with RML prions. Partial fixation and staining of the tissue prior to LCM extraction did not interfere with PMCA amplification. Studies are ongoing to analyze PrP<sup>Sc</sup> amplified from single cells extracted from infected brains by LCM. This technology will enable to study the characteristics of prions associated with different cell types isolated from distinct areas of the brain of animals and humans infected with different prion strains. Experiments can be done comparing PrP<sup>Sc</sup> in neurons versus other nerve cell types as well as different type of neurons and can be coupled with single cell genomics and proteomics analysis. These findings will shed light on the molecular basis of differential cellular vulnerability to prion infection and the nerve tropism of distinct prion strains.

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**P81 The nematode *C. elegans*: a model for therapeutic research in human prion diseases**

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*Caenorhabditis elegans* is a nematode with a well-defined neuronal system showing a strong gene homology with mammals including humans and devoid of human PrP homologs. It has proven to



be a powerful genetic model to study the molecular mechanisms of cell toxicity notably due to a gain of function mechanism and for medium to large scale therapeutic drug screens.

In a previous study, we showed that *C. elegans* model is appropriate to study the impact of mutated PrP on neuronal cell function. Here, we co-expressed human PrP harboring the E200K mutation, which is responsible for most cases of inherited Creutzfeldt-Jakob disease, and the GFP reporter fluorescent protein in the mechanosensitive neuronal system of the nematode. This sensitive system is involved in the detection of the environment of the animals. Its dysfunction can be detected and quantified by assessing the motility of the worms induced by the stimulation of the mechanosensitive reflex.

The expression of E200K PrP in mechanosensory neurons led to a progressive disorder associating a loss of touch response with a decrease in global motility and a reduced viability. Neurons expressing E200K PrP showed PrP-clustering and axonal blebbing. Moreover, animals from E200K PrP expressing line showed a significant loss of mechano-sensory neurons as compared to lines expressing wild type human PrP at similar levels. In addition, partially proteinase K-resistant form of PrP was detected in homogenate of E200K PrP expressing worms.

The objective of this study was to carry out, in E200K PrP expressing worms, a *non a priori* pharmacological screening of a large library of compounds that are both able to cross the blood-brain barrier and approved by the *FDA*. The first step relies on a high throughput screening using semi-automatized quantification of neuronal damages induced by E200K PrP expression. Molecules preventing neuronal cell death were further explored by assessing their effect on motility and locomotor reflex alteration. In addition, we analyzed the ability of the most promising compounds to modify the PrP aggregation process observed in E200K PrP line by quantifying the number and the volume of PrP-aggregates in the two posterior lateral microtubules neurons. Finally, the efficacy of the selected drugs to reduce PK-resistant PrP accumulation was assessed.

Here, we provide the first *in vivo* simplified genetic PrP model showing a specific prion-induced neuronal death. Our results validate the nematode *C. elegans* as a suitable model for therapeutic research. In addition to helping identifying new therapeutic targets, it will contribute to a better understanding of the relationship between PrP aggregation and neuronal loss.

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## **P82    *PRNP* sequence variation in British wild deer populations**

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Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy of cervids, originally identified in the USA. Uniquely among animal prion diseases, CWD is found in both captive and free ranging animals, posing significant implications for its control with the result that the disease is now widespread across North America. The identification of CWD in reindeer and moose in Norway since early 2016 raises concerns about the spread of disease in Europe, but relatively little is known about susceptibility of common European cervid species to CWD. Coding polymorphisms in the host prion protein gene (*PRNP*) are a key determinant of susceptibility to prion disease, therefore the aim of this project was to identify the most common *PRNP* alleles/sequences in the six major species of free-ranging deer found in the UK.

Samples from red, roe, sika, fallow, muntjac and Chinese water deer were obtained from existing DNA archives and ear-tip tissue sourced from hunters across the UK, providing a broad

geographical representation of the species. Following extraction of genomic DNA, we sequenced the open reading frame of *PRNP* from a total of 916 deer.

As for North American cervid species, the *PRNP* gene sequence found in British deer is well conserved between species. The most numerous and widespread species, roe deer, shows no *PRNP* genetic variation, while red deer express four different PrP variants, two of which have not been identified in North American species. The frequencies of *PRNP* alleles in red deer vary depending on geographic location. The effect of the corresponding amino acid substitutions on protein aggregation will be assessed using *in vitro* fibrillization assays. Our results show that in Great Britain, red deer have the greatest variation in *PRNP* sequence. The effect of the observed polymorphisms on susceptibility to CWD merits further investigation.

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### **P83 Diversity of Chronic Wasting Disease (CWD) strains in Canadian enzootic regions**

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Chronic wasting disease (CWD) is a contagious prion disease that occurs in various species of free-ranging and/or captive cervids in the United States (US), Canada, South Korea, Norway and Finland. CWD prions exist as multiple strains that produce different pathology and differ in their proclivity to infect hosts. *PRNP* allelic variants resulting in amino acid changes in the cellular prion protein (PrP<sup>C</sup>) can greatly influence the susceptibility of cervids to particular prion strains. We have shown that new prion strains can emerge following transmission between cervids expressing different *PRNP* alleles. Emergent strains can have novel transmission properties that enable them to infect hosts considered resistant to CWD, indicating that a diverse pool of circulating cervid prion strains is a concern for wildlife, agricultural and public health. We are determining the diversity of CWD strains responsible for CWD epizootics in Canada's wild and captive cervids. The strain properties of field CWD isolates (including a wt/G116 white-tailed deer isolate) and a pool of three experimentally-infected M132 homozygous elk are being characterized in various animal models. Elk CWD prions produced significantly different lesion profiles upon transmission in transgenic mice compared to other CWD isolates. In addition, S96-PrP<sup>C</sup> expressing mice infected with this agent accumulated PrP-res in brain in the absence of disease signs at the time of experiment termination. The A116G isolate caused clinical disease in mice expressing S96-PrP<sup>C</sup>. The PrP-res glycotypes produced by G116<sup>+</sup> and elk (CWD2) prions in these mice was novel. Our data indicate the existence of multiple CWD strains in wild deer and captive elk.

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### **P84 Study of the presence of infectivity in placenta from experimental Atypical scrapie infected sheep**

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Classical scrapie is the ovine TSE featured with a wide PrP<sup>Sc</sup> dissemination in many non-neural tissues and high levels of transmissibility within susceptible populations. Placentas from infected animals can accumulate a large amount of PrP<sup>Sc</sup> and play a key role in the horizontal transmission of the disease. In 1998 a new ovine TSE named Atypical or Nor98 scrapie was identified in Norway. In Atypical scrapie, PrP<sup>Sc</sup> seems to be restricted to central nervous system (CNS) and does not spread efficiently among sheep. However, Atypical scrapie can be transmitted experimentally to sheep and transgenic mice and infectivity has been detected in lymph nodes, peripheral nerves and muscles from sheep challenged with this strain. The aim of the present study is to investigate the presence of infectivity in placenta from Atypical scrapie animals.

Two 1 year old AHQ/AHQ sheep were intracerebral (IC) challenged with Atypical scrapie (1<sup>st</sup> passage). Animals were clinically checked twice a month and were euthanized when consistent neurological signs were observed (1118 and 1142 dpi). Brain from one of these animals was used to IC challenge four 3 month old lambs (2 AHQ/AHQ and 2 ARR/AHQ; 2<sup>nd</sup> passage). Clinical signs appeared at 514 and 689 dpi in AHQ/AHQ sheep and at 808 and 888 dpi in AHQ/ARR sheep. AHQ/AHQ sheep were euthanized in an advanced clinical phase of the disease (904 and 1173 dpi) but the AHQ/ARR sheep still remain alive (1788 dpi). At necropsy, samples from CNS and lymphoid tissues were collected and were analyzed for PrP<sup>Sc</sup> presence by IHC (8G8 antibody) and/or by IDEXX HerdChek scrapie/BSE Antigen EIA Test. PrP<sup>Sc</sup> was detected in CNS of all the animals but in none of the lymphoid tissues examined. A stronger signal of PrP<sup>Sc</sup> immunostaining and a wider PrP<sup>Sc</sup> dissemination in CNS was observed in AHQ/AHQ sheep from 2<sup>nd</sup> passage than from 1<sup>st</sup> passage. In these 2<sup>nd</sup> passage-cases, samples from retina, optic nerve and olfactory bulb were positive.

The inoculated sheep (from 1<sup>st</sup> and 2<sup>nd</sup> passage) were subjected to natural mating with a negative ram of the AHQ/AHQ genotype. At parturition, all AHQ/AHQ sheep were clinically affected. Placentas were collected and assayed for PrP<sup>Sc</sup> by IHC and/or IDEXX HerdChek scrapie/BSE Antigen EIA Test. No PrP<sup>Sc</sup> was detected in any of the placental samples examined. To test the presence of infectivity in these placentas, bioassays using transgenic mice overexpressing ovine PrP (Tg338) were performed. Six mice were IC inoculated with each placental sample and were monitored weekly. Mice were culled after  $639 \pm 36$  dpi without showing clinical signs and CNS samples were individually collected. Mouse samples were assayed for PrP<sup>Sc</sup> by PET-Blotting (SHa31 antibody) and all of them were negative.

The preliminary results from the present study support that atypical scrapie is not transmissible between individuals which is in accordance with the hypothesis that proposes this disease as a spontaneous disorder.

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## **P85 Molecular characterization of Scrapie cases in Spain**

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Small ruminants can be affected by at least two different types of Transmissible Spongiform Encephalopathies: classical scrapie and atypical scrapie. While Classical Scrapie was described in XVIII century, atypical scrapie was firstly described in Norway in 1998, although a retrospective study in United Kingdom discriminated a positive case in 1989. Since 2002, atypical scrapie has been identified in several countries worldwide including New Zealand, Japan, Canada and most of the European countries. There are some differences between classical and atypical scrapie including epidemiological and molecular characteristics like: sporadic distribution, diagnosed in aged animals, glycoform pattern of PrP<sup>Sc</sup> observed by Western blot, PrP<sup>Sc</sup> distribution in the central nervous system. Moreover, atypical scrapie affects sheep that carry prion protein genotypes associated with a relative resistance to classical scrapie, and there is rarely more than one animal per herd affected.

During the last ten years, the number of atypical scrapie cases detected in small ruminants in Spain remains constant. Since 2007, a total amount of 225 atypical scrapie outbreaks was informed, while the number of classical scrapie outbreaks was 125, although more than one positive animal can be found in a classical scrapie outbreak.

In this study, we describe the molecular characterization of atypical and classical scrapie isolates from the TSE control and eradication programme. Both, biochemical PrP<sup>Sc</sup> properties and epitope mapping with antibodies against different regions of PrP protein have been performed as well as genetic features and evaluation of gene expression profiles for cases affected of classical and atypical Scrapie.

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## **P86 Immunohistochemical characterization of BSE cases against different epitopes of prion protein**

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Bovine spongiform encephalopathy (BSE) is a transmissible spongiform encephalopathy of cattle detected around the world. Nowadays, the cases have decined but atypical BSE cases have been reported since soo4 in different countries. They have been classiffied as L and H type based on the differences in the molecular size of the PrP<sup>res</sup> unglycosylated protein band on Wstern blots compared to classical BSE (C-type).

Diffeences in PrP<sup>d</sup> immunoreactivity profiles between C-type, H-type an L-type has been reported in the literature; the ain of this study is to investigate the prP<sup>d</sup> deposition patterns by immunohistochemistry in Classical, and atypical (H-type and L-type) BSE cases using a panel of monoclonal antibodies against the upstream N-terminal, globular and C-terminal domains of the prion protein.

Samples from brainstem at obex level and cerebellun were analysed. Pre-treated sections with formic acid and hydrated autoclaved were used for histological (H&E) and immunohistochemicals techniques with prP monoclonal antibodies against different epitopes covering the PrP protein followed by an avidin-biotin peroxidase system. Immunoreactivity were observed employing a 3-3'-diaminobencidine according to validated methods for confirmatory test in our laboratory.

As previous studies has described (Casalone C. et al 2004, Ikada H. et al 2011, Simmons M. et al 2016), several PrP<sup>d</sup> profiles, extracellular-type, intracellular-type, were observed in C-type, H-type and L-type BSE cases. extracellular can be classified as linear, fine punctate, granular, coalescing or perineuronal, even more than one pattern at the same sample. Intracellular labelling was intra-neuronal and intra-glial.

Although the number of BSE atypical cases found are quite lower than BSE classical ones, PrP<sup>d</sup> immunolabelling allows to identify some striking different features between BSE cases analysed than can use to characterise this variants of the disease.

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### **P86B Antipion and antioxidant effects of carnosic acid and carnosol in a cellular model of prion diseases through KEAP1-NRF2 pathway activation**

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**Introduction:** Prion diseases are characterized by the structural conversion of the normal, cellular prion protein (PrP<sup>C</sup>) into an abnormally structured, partially protease-resistant isoform (PrP<sup>Sc</sup>). Although it is well established that PrP<sup>Sc</sup> is associated with disease, the actual molecular basis of prion diseases pathogenesis has not been determined in detail and no effective therapeutic intervention is currently available for prion diseases. Inhibition of PrP<sup>Sc</sup> formation has been a primary target towards this direction. Carnosic acid (CA) is a catechol-type electrophilic phenolic diterpene (CA, C<sub>20</sub>H<sub>28</sub>O<sub>4</sub>) with multiple bioactive properties including antioxidant, anti-inflammatory and antineoplastic properties. CA emerges as a promising candidate in the field of potential agents against neurodegenerative disorders. Both *in vitro* and *in vivo* studies suggest that CA exerts neuroprotection through activation of the Keap1-Nrf2 pathway. Carnosol (CS), a metabolite of CA, may also represent a potential therapeutic agent.

**Aim:** We aimed to evaluate CA and CS as neuroprotective/therapeutic agents against neurodegenerative disorders and in particular prion diseases.

**Materials and Methods:** We used murine neuroblastoma cells persistently infected with the 22L scrapie strain (22LN2a58), endogenously producing the aberrant PrP<sup>Sc</sup> isoform. Cells were treated with non-cytotoxic CA and CS doses; PrP<sup>Sc</sup> accumulation was assessed in treated cells relative to untreated cells and was expressed as the ratio of PrP<sup>Sc</sup> to total PrP determined by densitometric analyses of PK-treated and non PK-treated cell lysates respectively. Relative expression of the prion protein gene (*PRNP*) and of heme oxygenase 1 (*HO-1*), Nuclear factor erythroid-derived

2-like 2 (*Nrf2*) and Glutamate-cysteine ligase regulatory subunit (*GCL-M*), were estimated with qPCR.

**Results:** Effective concentration (EC<sub>50</sub>) corresponding to 50 µM was determined for both CA and CS, based on PrP<sup>Sc</sup> reduction at 48 hours following incubation with each compound. Immunoblotting analysis revealed that CA and CS used at the EC<sub>50</sub> concentration resulted in significant PrP<sup>Sc</sup> reduction after 48 hours treatment. Real-time PCR experiments demonstrated induction of *PRNP* gene expression in the presence of CA and CS as compared to controls (untreated cells). A significant induction of the transcription factor *Nrf2* and of the antioxidant enzymes *HO-1* and *GCL-M* genes was also observed in CA and CS treated cells.

**Conclusion:** We report an anti-prion effect of CA and CS in scrapie infected cells, associated with activation of the antioxidant Keap1-Nrf2 pathway and upregulation of *PRNP*. Our preliminary data indicates that oxidative stress may play an important role in PrP<sup>C</sup> to PrP<sup>Sc</sup> conversion and that *PRNP* expression might be regulated by Nrf2. Further analyses are in progress to study in more detail the underlying neuroprotective mechanisms.

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## **P87 Dual PrP<sup>res</sup> population of CH1641 is only maintained after transmission to TgOv mice**

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**Background.** CH1641 was originally isolated in the UK from a natural case of scrapie in a Cheviot sheep in 1970 and subsequently passaged intracerebrally in sheep, presumably carrying ARQ and/or AHQ alleles. CH1641 has been the subject of extensive research because of the similarity in the molecular size of the unglycosylated PrP<sup>res</sup> band (~19 kDa) in CH1641 and BSE infected sheep brain. In contrast to BSE however, CH1641 in sheep has the unique feature of a dual population of PrP<sup>res</sup> fragments (PrP<sup>res</sup> #1 and PrP<sup>res</sup> #2) which differ in molecular size after proteinase K digestion. We report here the transmission of experimental CH1641 to conventional and transgenic mice and the biochemical characteristics of PrP<sup>res</sup> from CH1641 in each mouse line.

**Methods.** The CH1641 brain homogenate (CH1641/4) used in this study was passaged 4 times by intracerebral inoculation in Cheviot sheep. Groups of 15-20 mice (RIII, VM, Tg338 (TgOv VRQ), Tg59 (TgOv ARQ) and Tg110 (TgBov) were inoculated intracerebrally with a 10% brain homogenate of CH1641/4. The brains of the mice were examined by PrP<sup>Sc</sup> profiling and triplex Western blot as reported previously (van Keulen et al, 2015)

**Results.** Transmission rates to RIII, Tg338, Tg59 and Tg110 mice were 100% whereas no transmission to VM mice occurred. Triplex Western blotting showed a low molecular unglycosylated band of ~19 kDa and a concomitant reduction of the N-terminal 12B2 epitope signal in all mouse lines comparable to sheep CH1641. However, the dual PrP<sup>res</sup> population characteristics of sheep CH1641 was only maintained in the TgOv mice whereas no dual PrP<sup>res</sup> population was observed in the RIII and Tg110 mice. Cross transmission of CH1641 from RIII and Tg110 into Tg 59 mice resulted in the re-appearance of the dual PrP<sup>res</sup> population. In addition, the resulting immunohistochemical PrP<sup>Sc</sup> profile was similar to the profile from the direct transmission of CH1641 into Tg59 indicating that the same strain had been isolated in all mouse lines.

**Conclusion.** The dual PrP<sup>res</sup> population of sheep CH1641 is only maintained on transmission into TgOv mice and not into conventional mice or TgBov mice. This raises questions as to whether CH1641 could be present in other animal species showing the low molecular 19kDa unglycosylated PrP<sup>res</sup> band but without the characteristic dual PrP<sup>res</sup> population thus mimicking C-type BSE.

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## **P88 Characterization of gene expression of Toll-like receptors 1–10 in selected tissues from naturally infected sheep**

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In the last decade, a growing of evidence indicates that common pathological phenotypes found in neurodegenerative disorders could be explained by uncontrolled chronic immune and inflammatory responses. In these disorders characterized by accumulation of misfolded proteins, such as Alzheimer, it is described an immune response mediated by toll like receptors (TLRs). These receptors are a type of pattern recognition receptors that upon activation by either exogenous or endogenous ligands trigger protective responses during the early stages of infection (innate response), and also exert a profound influence on the generation of the adaptive immune responses. However, it is demonstrated that the continue stimulation of the innate response might lead to the pathological effect. The question raised is whether the misfolded prion protein might act as a ligand of these receptors and, in turn, trigger a protective or harmful response in the host. Prion disorders do not display any clear inflammatory infiltrates or produce antibodies to the prion protein, whereas there is an activation of glial cells and the involvement of lymph reticular system (LRS) in their pathogenesis. In central nervous system (CNS), the innate immunity basically relies on glial cells, therefore it is not unlikely that the glial activation can be reconciled with TLR activation. Furthermore, experimental *in vitro* and *in vivo* studies revealed that recombinant prion preparations can up-regulate certain TLRs and downstream signaling molecules.

We studied the expression profiles of TLRs in the spleens of naturally scrapie infected sheep compared to non-infected animals and then other target tissues, nervous system and digestive tract, will be further analyzed. 5 scrapie infected sheep, euthanized at advanced clinical stage, and 5 paired control sheep were selected. At necropsy, samples of various areas from CNS, LRS and ileum were collected and the scrapie status was confirmed by the immunohistochemically detection of PrP<sup>Sc</sup> in these tissues. Quantitative real time PCR assays confirmed expression of all 10 TLRs and 3 reference genes (*GAPDH*, *ACT-β* and *SDHA*). To determine any statistically significant differences between groups the Mann-Whitney U non-parametric test and t-student parametric test were used (SPSS v.24, IBM Corp., Armonk, NY, USA). Preliminary results of all spleen samples analyzed, showed no significant differences in the transcriptional expression of each of the 10 TLRs between infected and control animals; yet the *TLR10* showed a tendency to be higher expressed in infected sheep compared to control sheep (*p-value* 0.099). In agreement with previous studies in ovine lymphoid tissues, *TLR6*, *TLR7* and *TLR10* were the most abundant of the TLRs in spleen. The ongoing studies will analyze the TLRs expression in other target tissues and at different stages of the disease to have an improved understanding of a potential participation of these TLRs in the scrapie pathogenesis.

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**P89 Preclinical detection of prions in skin samples of scrapie-infected animals by serial PMCA and RT-QuIC assays**

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See O11

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**P90 Macaque and human PrP<sup>C</sup> exhibit different prion strain susceptibility in transgenic mice**

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Macaques have been used as a relevant model for the evaluation of the zoonotic potential of prion diseases, especially for classical-BSE infectious agent. PrP amino acid (aa) sequence plays a key role in prion susceptibility. Macaque and human PrP sequences have a high degree of homology (96.4%) with only nine aa differences, but it is known that one aa difference could be sufficient to alter prion susceptibility. In order to evaluate the effect of those aa changes in prion susceptibility, the transmissibility of a panel of diverse prions from different species (human, cattle and sheep) has been compared in transgenic mice expressing either macaque or human PrP<sup>C</sup>.

A panel of isolates representing different prion strains have been intracranially inoculated in transgenic mice expressing either macaque (TgMac) or the Met 129 variant of human PrP<sup>C</sup> (Hu-Tg340). Both mouse lines express the transgenic PrP<sup>C</sup> under the control of the murine *prnp* gene regulatory sequences on PrP-knockout backgrounds.

Transmission of human prions in TgMac mice was only positive in the case of vCJD isolate, showing similar survival time than Hu-Tg340 vCJD inoculated mice. Atypical BSE-L infected both TgMac and Hu-Tg340 mice with 100% attack rates and similar long survival times. None of



the mice inoculated with atypical BSE-H in both mouse lines was scored positive for the disease. It is interesting to remark the case of classical-BSE which was more efficiently transmitted in TgMac than in Hu-Tg340 mouse line. Partial transmission was observed for one of the scrapie isolates inoculated in TgMac mice while none of the Hu-Tg340 was scored positive. These experiments evidenced some differences in the susceptibility of these two transgenic mouse models to certain prion isolates. In particular, transgenic mice expressing macaque PrP were more susceptible to BSE strain infection than those expressing human PrP.

These results suggest that, the nine amino acid differences between macaque and human may have an important effect on prion susceptibility depending on the prion strain, allowing us to confirm that strain properties (probably associated to PrP<sup>Sc</sup> conformers) have a determinant impact on the species barrier. Taken together these results suggest that macaque is an adequate model for the evaluation of human susceptibility to most of the prion strains tested here (vCJD, BSE-L, BSE-H, etc.). The differential susceptibility to classical-BSE transmission between macaque and human PrP<sup>C</sup> should be taken into account for the interpretation of the results obtained in macaques inoculated with BSE. It could notably explain why the macaque model turned out to be so efficient (worst case model) until now to model human situation towards BSE despite the limited number of animals inoculated in the laboratory experiments.

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#### **P91 Highly sensitive detection of PrP<sup>Sc</sup> derived from various prion strains by simultaneous addition of digitonin, heparin and arginine ethyl ester to protein misfolding cyclic amplification**

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The presence of abnormal prion protein (PrP<sup>Sc</sup>) in specimen is an indicator for the diagnosis of prion diseases. Protein misfolding cyclic amplification (PMCA) is a technique to rapidly amplify the minute amounts of PrP<sup>Sc</sup> more than 10 billion times *in vitro*, and enables highly sensitive detection of PrP<sup>Sc</sup>. However, the efficiency for PrP<sup>Sc</sup> amplification differs drastically among various “prion strains”, which exhibit distinct disease phenotypes in identical hosts. The low amplification efficiency for certain prion strains is a practical problem in applying PMCA as a diagnostic tool for prion diseases. Here, we investigated whether amplification of PrP<sup>Sc</sup> from various strains can be enhanced by adding known amplification promoting factors alone or in combinations. Addition of heparin and digitonin not only made it possible to amplify PrP<sup>Sc</sup> from multiple prion strains but also increased amplification efficiency of all of 9 prion strains other than L-BSE. Furthermore, addition of arginine ethyl ester (ArgEE) with them allowed efficient amplification of L-BSE PrP<sup>Sc</sup> and further highly sensitive detection of PrP<sup>Sc</sup> from chronic wasting disease and scrapie. These results suggest that the simultaneous addition of digitonin, heparin and ArgEE increases the sensitivities for detection of PrP<sup>Sc</sup> by PMCA regardless of prion strains, and this simple and improved PMCA may be applicable for the early diagnosis of prion diseases and for basic research such as species barriers and mechanism for the generation of prion strains.

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**P92 Reduction of Microglia in Brain with the CSF1R inhibitor PLX5622 Accelerates the Tempo of Prion Disease**

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Microglial cells in the central nervous system play important roles in neurodevelopment and resistance to infection, yet microglia can become neurotoxic under some inflammatory conditions. An early event during prion infection is the activation of microglia and astrocytes in the brain prior to damage or death of neurons. The influence of microglia in prion-induced neurodegeneration is unclear and results from previous studies are conflicting. In vitro exposure of neurons to the prion protein fragment PrP<sub>106-126</sub> is toxic, and microglia are required for this neurotoxicity in culture. More recently, a neurotoxic role for microglia during prion infection was proposed in studies where scrapie-infected mice were orally treated with CSF1R inhibitor, GW2580. In contrast to the above studies, microglial depletion in cerebellar organotypic slice cultures resulted in a small, but detectable, increase in PrP<sup>Sc</sup> deposition and an increase in prion infectivity titers. These data suggest microglia may be able to clear prions from infected tissues. Furthermore, scrapie infection in IL-34-deficient mice, with 50% fewer microglia than normal, showed decreased survival compared to wild-type mice. These results suggest that microglia may be neuroprotective during prion disease. To directly address the contribution of microglia during prion infection, we chemically ablated microglia from mice using the CSF1R tyrosine kinase inhibitor PLX5622. Depletion of microglia in the CNS early in prion infection significantly accelerated prion disease progression, astrogliosis, and spongiform change with three scrapie strains. Mice in which microglia were ablated were euthanized 20 to 32 days earlier than infected control mice due to advanced clinical scrapie. Moreover, treatment with PLX5622 at 80 days post-infection (preclinical) also accelerated disease and resulted in euthanasia of mice 33 days earlier than infected controls. Analysis of transcripts from isolated thalamus or whole brain of treated mice showed reduction in several proinflammatory genes, suggesting microglia directly or indirectly contribute to expression of these genes during prion infection. These studies indicate that microglia play a critical protective throughout prion disease and may be most effective in the later stages.

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**P93 The agent of chronic wasting disease from pigs is infectious in transgenic mice expressing human *PRNP***

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**See WA15**

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## **P94 Prion substrain selection and lymphotropism influenced by PrP<sup>C</sup> expression levels**

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Prions are formed from abnormal conformers (PrP<sup>Sc</sup>) of the host-encoded cellular prion protein (PrP<sup>C</sup>). They replicate by templating the conversion and polymerization of PrP<sup>C</sup> by an autocatalytic process. Multiple strains of prions are recognized phenotypically within the same host species. Strains are conformational variants of PrP<sup>Sc</sup> at the level of the tertiary and/or quaternary structure. In infected hosts, prion strains exhibit stereotyped phenotype, including specific tropism for the cerebral and lymphoid tissues. Extraneural replication in the lymphoid tissue is not mandatory but increases the disease penetrance.

There is clear evidence that prions, be natural isolates or experimental sources, can be composed of several substrains, although one dominant PrP<sup>Sc</sup> conformation is usually detected by conventional immunodetection methods. Thus, homotypic transmissions can lead to seemingly different outcome due to preferential selection of one substrain, as observed during heterotypic transmission with species barrier.

By studying intracranial transmission of natural sheep scrapie isolates (termed LAN) to transgenic mice expressing different levels of homologous PrP<sup>C</sup> in the brain, we demonstrated that prion substrain selection can be governed by PrP<sup>C</sup> amount (1). Thus, one of the subcomponent of LAN field isolates so-called LA19K was preferentially selected on 'high-expressor' mice, whereas the so-called LA21K dominant component was selected in mice expressing near physiological PrP<sup>C</sup> levels. In independent studies, we also reported that transmission of human prions, be it in a homotypic context or not, could led to isolation of different strain types in the brain and spleen tissue of the same mouse (2-4).

As PrP<sup>C</sup> levels markedly differ between brain and spleen, these observations, collectively, led us to hypothesize that tissue PrP<sup>C</sup> amounts could be a key determinant of prion substrain selection within the same individual. Therefore, we examined the outcome of LAN isolates transmission in the brain and spleen tissue of high-expressor mice. In accordance with their replication rate dependency on PrP<sup>C</sup> amount, we show preferential selection of LA19K and LA21K prions in the brain and spleen tissue, respectively. We also show that the route of infection impacts which strain type is preferentially selected, the intraperitoneal route allowing neuropathological expression of LA21K prions. Reversely, we show that a pair of strains that co-propagates in high-expressor mice, albeit in different tissue (T1<sup>Ov</sup> in spleen and T2<sup>Ov</sup> in brain (4)) showed opposite tropism in low-expressor mice (T1<sup>Ov</sup> in brain).

Altogether, our results support the view that PrP<sup>C</sup> expression levels are instrumental in prion substrain selection and specific tropism for the lymphoid tissue.

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2. Béringue *et al. Science* **335**, 472-475 (2012).
3. Béringue *et al. PLoS One* **3**, e1419 (2008).
4. Chapuis *et al. Acta Neuropathol Commun* **4**, 10 (2016).

**P95 Detection of seeding activity in preclinical blood samples from BSE-infected sheep using protein misfolding cyclic amplification (PMCA)**

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**P96 Reliable identification of different strains in caprine scrapie isolates by a PrP<sup>Sc</sup>-based approach**

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Introduction

Evidences on sheep and goat susceptibility to BSE and the discovery of natural BSE infection in 2 goats prompted the European Commission to improve BSE surveillance in small ruminants. Although the BSE agent can be recognized by biologic strain typing in mice, large scale testing required molecular tests able to discriminate BSE from the most common small ruminant TSEs. Whilst current discriminatory tests focus on distinguishing the BSE agent from all others, they cannot discriminate among classical scrapie strains. Still, classical scrapie can be caused by various TSE strains with different PrP genotype targets and biological features. In Italy, scrapie isolates showed uniform biochemical and biological properties so far, suggesting the circulation of a single or largely prevalent strain. We developed an improved discriminatory method able to discriminate Italian scrapie isolates from other European isolates. Here we report a proof-of-principle application of this method to active surveillance, which allowed to identify a non-Italian strain in 3 imported scrapie cases.

Methods

In 2015, the Italian active surveillance identified a scrapie outbreak in goats imported from Romania. Three isolates from this outbreak were analyzed by official discriminatory WB protocol. These 3 isolates were also analyzed by the improved ISS discriminatory WB, which relies on strong PK treatment (1 mg/ml). The SAF84/P4 ratio allows to categorize scrapie samples in 2 groups: P4-res with lower mAbs ratio (<0.6) and P4-sen with higher mAbs ratio (>0.6). We further investigated the biological properties of scrapie isolates by bank vole bioassay.

Results

By the official discriminatory WB protocol, the 3 imported isolates showed a homogeneous molecular pattern, characterized by ~18 kDa unglycosylated PrP<sup>res</sup> and a glycoform ratio similar to that observed in Italian scrapie isolates; however, all showed a slightly lower SAF84/P4 ratio compared to the usual pattern of Italian cases. Accordingly, the improved discriminatory WB allowed to discriminate the 3 imported cases from other Italian isolates, with SAF84/P4 ratios of

~0.4 and >0.6, respectively. Overall, the 3 isolates from imported goats showed biochemical characteristics different from those of the Italian isolates. Importantly, bioassay in voles confirmed that the strain involved was the same in the 3 imported cases and different from the strain circulating in the Italian sheep and goat populations.

#### Conclusions

Active surveillance needs large scale testing, possibly with fine biochemical methods able to detect strain variation in field isolates. We showed that the improved ISS discriminatory WB allowed to reliably identify scrapie strain variation and to prevent the spread of a new strain in Italy. Reliable molecular discrimination might increase the specificity of TSE surveillance and reduce the requirement for bioassay.

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#### **P97 Serial detection of hematogenous prions in CWD-infected deer**

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Chronic wasting disease (CWD) studies in the native white-tailed deer host— spanning fifteen years— have provided a unique repository of serially collected samples. This repository has permitted us the opportunity to evaluate intra- and inter-host CWD trafficking, dissemination, and transmission throughout the protracted disease course. We have extended our previous studies to examine hematogenous prion load in blood collected minutes, days, weeks and months post exposure, by employing enhanced amplification methodologies. Here we corroborate our previous findings, by detecting amyloid formation in whole blood and blood cell fractions harvested as early as 15 minutes post inoculation, throughout the disease course, peaking at clinical disease. These findings bring the prion field a step closer to characterizing hematogenous prions and their role in the spread of prion disease.

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#### **P98 The agent of H-type bovine spongiform encephalopathy associated with E211K prion protein polymorphism transmits after oronasal challenge**

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In 2006, a case of H-type bovine spongiform encephalopathy (BSE) was reported in a cow with a previously unreported prion protein polymorphism (E211K). The E211K polymorphism is heritable and homologous to the E200K mutation in humans that is the most frequent PRNP mutation associated with familial Creutzfeldt-Jakob disease. Although the prevalence of the E211K polymorphism is low, cattle carrying the K211 allele develop H-type BSE with a rapid onset after experimental inoculation by the intracranial route. The purpose of this study was to investigate whether the agents of H-type BSE or H-type BSE associated with the E211K polymorphism transmit to wild type cattle or cattle with the K211 allele after oronasal exposure. Wild type (EE211) or heterozygous (EK211) cattle were oronasally inoculated with either H-type BSE from the 2004 US H-type BSE case (n=3) or from the 2006 US H-type case associated with

the E211K polymorphism (n=4) using 10% w/v brain homogenates. Cattle were observed daily throughout the course of the experiment for the development of clinical signs. At approximately 50 months post-inoculation, one steer (EK211 inoculated with E211K associated H-BSE) developed clinical signs including inattentiveness, loss of body condition, weakness, ataxia, and muscle fasciculations and was euthanized. Enzyme immunoassay confirmed that abundant misfolded protein was present in the brainstem, and immunohistochemistry demonstrated PrP<sup>Sc</sup> throughout the brain. Western blot analysis of brain tissue from the clinically affected steer was consistent with the E211K H-type BSE inoculum. With the experiment currently at 55 months post-inoculation, no other cattle in this study have developed clinical signs suggestive of prion disease. This study demonstrates that the H-type BSE agent is transmissible by the oronasal route. These results reinforce the need for ongoing surveillance for classical and atypical BSE to minimize the risk of potentially infectious tissues entering the animal or human food chains.

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### **P99 Influence of cross-species transmission on the host range of CH1641-like scrapie isolate**

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**Introduction.** In prion diseases, a disease-associated isoform of prion protein (PrP<sup>d</sup>) is accumulated in the brain of affected animals. Scrapie in sheep and goats has been hypothesized to be a candidate for the origin of BSE in cattle since the discovery of the first BSE case. However, cattle inoculated with typical classical scrapie isolates never developed disease phenotype of BSEs, including atypical BSE. CH1641 scrapie isolate established in the UK is characterized by a lower molecular mass of the unglycosylated form of the protease-resistant core of PrP<sup>d</sup> (PrP<sup>res</sup>) compared to that of typical classical scrapie isolates. A few natural CH1641-like scrapie cases have been found in Europe. However, no transmission studies to cattle have been reported to date. We also established CH1641-like experimental scrapie isolate (designated as Sh294). Sh294 cannot transmit to wild-type mice like the British CH1641 scrapie isolate. But it can transmit to bovine PrP overexpression (TgBoPrP) mice. Interestingly, these mice showed similar clinical signs of the disease with L-BSE infection. Therefore, we first investigated the biological and biochemical properties of Sh294 in TgBoPrP mice to reveal the relationship between CH1641-like scrapie and L-BSE. Then, we attempted to transmit Sh294 passaged in TgBoPrP mice (designated as TgBo-passaged Sh294) to wild-type mice because prions sometimes change their host range by the interspecies transmission.

**Materials and Methods.** Sh294 was intracerebrally inoculated and serially passaged in TgBoPrP mice. Wild-type (ICR) mice were also intracerebrally inoculated with TgBo-passaged Sh294.

**Results.** Western blot (WB) analysis demonstrated similar PrP<sup>res</sup> banding patterns between TgBoPrP mice infected with Sh294 and L-BSE. However, the survival periods of mice infected with L-BSE were significantly shorter than those of mice infected with Sh294 even at the 3<sup>rd</sup> passage ( $144 \pm 4.8$  days vs  $177 \pm 4.5$  days). Immunohistochemical analysis also demonstrated the different PrP<sup>d</sup> distributions between them. To have a much better understanding the difference between Sh294 and L-BSE, cross-species transmission of TgBo-passaged Sh294 to ICR mice was conducted. In contrast with L-BSE, PrP<sup>res</sup> accumulation was confirmed by WB analysis in seven

out of twenty mice sacrificed at the end of their lifespan ( $807 \pm 46.9$  days). Subsequent transmission to ICR mice resulted in shortened mean survival periods ( $453 \pm 40.4$  days at the 3<sup>rd</sup> passage) with 100% attack rate. CH1641 scrapie specific PrPres banding patterns were detected in the brain of ICR mice by WB analysis.

Conclusion. We here indicate that CH1641-like scrapie isolate (Sh294) could be independent of classical and atypical BSEs. In addition, we succeeded in the first stable reproduction of the CH1641-specific PrPres banding patterns in wild-type mouse model. These data imply that the interspecies transmission to cattle may affect the host range of CH1641 scrapie prion.

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#### **P100 Vaccination of transgenic elk PrP mice and reindeer with recombinant prion protein overcomes self-tolerance and protects mice against CWD infection**

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See WA17

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#### **P101 Identification and transcriptome analysis of brain regions vulnerable to neuronal loss in prion infection**

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Prion diseases are fatal neurodegenerative disorders characterized by the accumulation of disease-associated form of prion protein (PrP<sup>Sc</sup>), activation of astrocytes and microglia, and vacuolation of neurons and neuropils in central nervous system. Neuronal loss is also often observed, but molecular mechanisms remain to be elucidated. In order to clarify the mechanism for neurodegeneration in prion diseases, in the current study, first we identified brain regions where neuronal loss was observed in mice infected with prion Chandler and Obihiro strain, and then analyzed transcriptome of the region by RNA-sequencing. When we analyzed the region where neuronal loss occurs by staining frozen section with anti-NeuN antibody, neuronal loss was observed in the thalamus at 120 and 145 days post inoculation (dpi). In thalamus, a higher level of PrP<sup>Sc</sup> accumulation was observed compared to other regions at 90 and 120 dpi. Microglia appeared to be more activated in thalamus than in other regions while astrocytes appeared to be less activated despite the high PrP<sup>Sc</sup> level. To analyze changes associated with neuronal loss, we performed comprehensive gene expression analysis of lateral dorsal part of the thalamus where progressive neuronal loss was observed. Among the differentially expressed genes, 58% of up-regulated genes were microglia-enriched genes, while 29% of down-regulated genes were neuron-enriched genes. Next we selected 2,222 neuron-enriched genes and analyzed differentially expressed gene. Several genes of neurotransmitter receptors, such as metabotropic glutamate receptors, GABA receptors, and muscarinic acetylcholine receptors, were found to be down-regulated. Of particular interest, the expression of several genes that are known to be up-regulated under the unfolding protein response was up-regulated. Given that apoptosis is induced by unfolding protein response and that unfolding protein response occurs in brains of animals

affected by prion diseases, the up-regulation of those genes in neuron-enriched genes suggests that unfolding protein response induced in neurons may be one of the mechanisms for neuronal loss in the thalamus of prion-infected mice.

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#### **P102 Polymorphism in PrP and its relevance for moose and public health**

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A present missense mutation in the *PRNP* gene of Scandinavian moose leads to a change of lysin (K) to glutamine (Q) in amino acid position 109, which may affect the proteolytic processing of PrP. Amino acid variations at this critical position in PrP could potentially influence pathology, disease susceptibility, incubation time, and disease transmission between species, if the mutated PrP is transformed into prions. We have investigated allele- and genotype frequencies of this potential harmful mutation in past and present Swedish moose populations. We have also investigated the protein cleavage pattern to elucidate potential associations between the polymorphism and moose health. In moose affected by Moose Wasting Syndrome (MWS), Cu and Zn levels are known to be severely altered, and research has shown that major cleavage sites of PrP<sup>C</sup> may vary in response to Cu<sup>2+</sup> and Zn<sup>2+</sup>. Therefore, we further studied the association between proteolytic pattern, metal ion level, genotype and MWS. Our working hypothesis is that small changes in a PK-sensitive prion protein could be associated with health condition in wild and domestic ungulates. Moreover, mutations that introduce small changes in prion protein sequence, and are spread in a population, could over time increase the risk of induction of a prion strain. The clinical picture and transmissibility may then vary, depending on the type of previous PrP mutation. This could possibly be of concern, especially considering the unknown background of the recent outbreak of Chronic Wasting Disease (CWD) in a wild reindeer herd as well as single cases in moose, and red deer in Norway. Recently, CWD has also been reported in moose in Finland.

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#### **P103 Oral transmission of CWD into *Cynomolgus* macaques: signs of atypical disease, prion conversion and infectivity in macaques and bio-assayed transgenic mice**

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See WA2

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**P104 Absence of Classical and Atypical (H- and L-Type) BSE Prions in the Blood of Clinical Bovine Cases as Confirmed by Cattle to Cattle Blood Transfusions**

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**See WA11**

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**P105 Evolution of Chronic Wasting Disease Prion Conformers**

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**See WA4**

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**P106 A scrapie cohort study shows the success of breeding for resistance**

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**See WA12**

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**P107 Pig-PrP as a paradigm of resistance to prion propagation**

Espinosa JC (1), Aguilar-Calvo P (1), Marín-Moreno A (1), Pitarch JL (1), Prieto I (1), Benestad SL (2), Andréoletti (3) O & Torres JM (1)

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**See WA9**

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**P108 PMCA as a platform for pre-symptomatic CWD diagnosis. Validation in field samples**

Morales R (1), Kramm C (1,2), Nichols T (3), Lyon A (1), Gomez-Gutierrez R (1,4), Pritzkow S (1) and Soto C (1)

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**See WA8**

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**P109 Caprine BSE and scrapie discrimination and scrapie strain identification by using Tgshp IX mouse bioassay**

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It is well known that classical scrapie in sheep shows a strain variation exhibiting distinct disease phenotypes. Results published in the last years clearly indicate that the same is true for classical scrapie in goats. Additionally, in contrast to sheep, two natural BSE infections have been proven in goats.

A few years ago, we characterized the diversity of natural scrapie field cases from Cyprus goats. In doing so, differences between single animals concerning the spread of the pathological prion protein (PrP<sup>D</sup>) in peripheral tissue samples as well as deviations observed in the biochemical pattern of brain samples became obvious. However, it remained unclear to what extent these characteristics might reflect a strain diversity. Therefore, nine representative brain stem isolates from these classical goat scrapie field cases (eight wild type and one R154H goat) as well as two isolates from experimental goat BSE cases (one wild type and one R211Q goat) were intracerebrally inoculated into Tgshp IX mice, which overexpress the wild type ARQ allele of the ovine PrP.

All isolates were in first passage successfully transmitted to Tgshp IX mice with high efficiency and incubation periods from 272 to 585 days post infection. The phenotypic characterization of the mouse brains revealed at least three different groups of scrapie isolates, clearly demonstrable by immunohistochemistry. One of these phenotypes is associated to the R154H goat. Additionally both BSE isolates showed a similar phenotype, which can clearly be discriminated from the scrapie isolates. Furthermore, the phenotypic differences seen in mice reflected in parts the differences seen in the natural host.

In conclusion, the Tgshp IX mouse bioassay is a useful tool to discriminate between BSE and Classical scrapie cases. Additionally it allows the isolation of different scrapie strains from natural cases, suggesting a similar strain variability in the field as described for sheep scrapie. Furthermore the results indicate that strain diversity might be responsible for variabilities in the biological properties of PrPD seen in peripheral tissue samples of the natural host. Hence, findings reported here are important for the understanding of classical scrapie pathogenesis in goats.

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### **P110 Using mass spectrometry to determine the relative susceptibility of PrP polymorphisms to atypical scrapie**

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A novel form of scrapie was described in 1998 and referred to as Nor98 for the country of origin and date of its discovery. Since then it has been found in numerous countries, including New Zealand and Australia, and has been renamed atypical scrapie. Unlike classical scrapie, the epidemiology of this sheep prion (PrP<sup>Sc</sup>) disease is consistent with a sporadic origin. Even though it may arise spontaneously, atypical scrapie can be experimentally transmitted to other sheep. Atypical scrapie is associated with specific PrP<sup>C</sup> polymorphisms that are different from those associated with classical scrapie. We used a mass spectrometry-based method to determine the relative amount of each PrP polymorphism present in a sample from a heterozygous animal. The total amount and relative amounts of each PrP polymorphism present in PrP<sup>Sc</sup> and PrP<sup>C</sup> were determined. Each PrP sample was isolated and digested with chymotrypsin to yield a set of characteristic peptides spanning relevant polymorphisms at positions 136, 141, 154, 171 and 172 of sheep PrP<sup>C</sup>. <sup>15</sup>N-labeled internal standards, derived from chymotrypsin digested <sup>15</sup>N-labeled rPrP, were used to quantify PrP polymorphisms (ALRRY and ALHQY or ALRQD or AFRQY) present in heterozygous atypical scrapie-infected or uninfected control sheep. Full length and truncated (C1) natively expressed PrP<sup>C</sup> isolated from atypical scrapie-infected animals showed both PrP polymorphisms are produced in equal amounts. In addition, similar amounts of PrP<sup>C</sup> are present in either infected or uninfected animals. The amount of PrP<sup>Sc</sup> isolated from infected heterozygotes was variable, but was composed of significant amounts of both PrP polymorphisms, including the ALRRY polymorphism which is highly resistant to classical scrapie. Atypical scrapie infection does not originate from sheep PrP<sup>C</sup> overexpression. Atypical

scrapie prions replicate at comparable rates, in spite of polymorphisms at positions 141, 154, 171, or 172.

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#### **P111 Sequence Analysis of Prion Protein Gene of Pakistani sheep breeds from four Provinces**

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Prions are considered the only agents of transmissible spongiform encephalopathies (TSEs) and are harmful pathogens of mammals. These infectious agents of host are made up through aggregation of conformational isomers (PrP<sup>Sc</sup>) and encode glycoprotein (PrP<sup>C</sup>) of 33-35 kDa. TSEs are the fatal group of diseases which are neurodegenerative and include chronic wasting disease in deer and elk, Creutzfeldt-Jakob disease (CJD) and transmissible mink encephalopathy (TME) in humans and scrapie in goats and sheep. The accumulation of abnormal form of the normal protein (PrP) is common in all diseases related TSE. This abnormal form of PrP called PrP<sup>Sc</sup> is resistant to proteolysis as well as infectious. Present study was conducted in order to do sequence analysis of prion protein gene in twelve breeds of the sheep. We studied this gene to elucidate 12 of Pakistani sheep breeds and to compare gene order with other mammalian species. PCR amplification of 771 bp fragment was done on selected samples from all twelve breeds followed by sequencing. Sequence analysis was done and some sites were found to be heterozygous. These findings on prion protein gene in sheep will provide assistance for further studies on pathogenesis, cross-species transmission, breeding programs, resistance and susceptibility to scrapie.

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#### **P112 Sequence analysis and polymorphism of Prion Protein Genes of Pakisani Goat breeds from four provinces including AJK**

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The association between caprine PrP gene polymorphisms and its susceptibility to scrapie has been investigated in current years. As the ORF of the PrP gene is extremely erratic in different breeds of goats, we studied the PrP gene polymorphisms in 80 goats which belong to 11 Pakistani indigenous goat breeds from all provinces of Pakistan. A total of 6 distinct polymorphic sites (one novel) with amino acid substitutions were identified in the PrP gene which includes 126 (A -> G), 304 (G -> T), 379 (A -> G), 414 (C -> T), 428 (A -> G) and 718 (C -> T). The locus c.428 was found highly polymorphic in all breeds as compare to other loci. On the basis of these PrP variants NJ phylogenetic tree was constructed through MEGA6.1 which showed that all goat breeds along with domestic sheep and Maufon sheep appeared as in one clade and sharing its most recent common ancestors (MRCA) with deer species while Protein analysis has shown that these polymorphisms can lead to varied primary, secondary and tertiary structure of protein. Based on these polymorphic variants, genetic distance, multidimensional scaling plot and principal

component analyses revealed the clear picture regarding greater number of substitutions in cattle PrP regions as compared to the small ruminant species. In particular these findings may pinpoint the fundamental control over the scrapie in *Capra hircus* on genetic basis.

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### **III. A $\beta$ , tau, synuclein, SOD1, TDP43, FUS...and other prion and/or prion-like proteins causing human disease**

#### **P113 Differentiation between $\alpha$ -synuclein strains in PD and DLB through Real Time-Quaking Induced Conversion**

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Synucleinopathies are a class of neurodegenerative diseases which include, among others, Parkinson's Disease (PD) and Dementia with Lewy Bodies (DLB), characterized by the presence of insoluble  $\alpha$ -synuclein aggregates. Recent evidences are indicating that  $\alpha$ -synuclein might behave as a prion-like protein, being able to misfold into a  $\beta$ -sheet enriched, pathogenic form, capable of contact and convert its native counterpart into the pathogenic variant, thus producing a positive feedback that will eventually cause the aggregation into Lewy bodies and neurites, hallmark structures of  $\alpha$ -synucleinopathies.

The Real time-Quaking Induced Conversion (RT-QuIC) assay is a technique that exploits the seeding-conversion mechanism of the prion and prion-like proteins to amplify tiny amounts of misfolded, proteopathic seeds by letting them reacting with its native counterpart through a series of cycles of incubation and shaking. The kinetic of aggregation is monitored in real time by thioflavin dyes, a class of fluorophore that binds to  $\beta$ -sheets enriched structures.

Here, we took advantage of the RT-QuIC to diferentially detect the seeding-conversion activity of pathogenic  $\alpha$ -synuclein derived from fontal cortex and substantia nigra pars compacta of PD and DLB patients. Our results, validated through PK digestion followed by Western Blot and dot blot for fibrillar synuclein, showed a different seeding activity between PD and DLB, possibly pointing to a strain difference between the two pathologies.

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#### **P114 Abnormal processing of SINE non-coding RNAs in amyloid pathology**

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As the human life span increases, the number of people in Canada suffering from dementia is expected to rise dramatically. In a previous work we have shown that learning impairment in dementia is connected with hippocampus-specific changes of gene expression including Immediate Early Genes (IEGs) and microRNAs, (Peleg\*, Sanabenesi\*, Zovoilis\* et al, Science 2010; Zovoilis et al, EMBO J 2011). However, the molecular mechanisms associated with this gene expression deregulation remain largely unknown. Among mechanisms that have recently attracted attention are those involving non protein-coding RNAs (non-coding RNAs), including RNAs derived by repetitive DNA (Zovoilis et al, Cell 2016). Repetitive DNA accounts for ~50% of the noncoding sequences, with Short Interspersed Nuclear Elements (SINEs), being among the most frequent repeats. Recently, we showed that in mouse, RNA from one of the most frequent SINE repeat classes (B2 RNA) controls expression of immediate early genes (IEGs) through binding and inhibition of RNA Polymerase II. In this way, IEGs remain poised for activation by being occupied by an elongating RNA Polymerase II that is transiently blocked by SINE RNAs and get released only upon a stimulus that leads to RNA processing of the inhibiting SINE RNA. However, it remains unknown whether there are any pathological conditions connected with deregulation of SINE RNA processing. Here, we applied an integrative RNA genomics and bioinformatics approach to dissect any connection between RNA processing and amyloid pathology in Alzheimer's disease mouse models and patients. Our study reveals that SINE RNAs are subject to RNA processing both in mouse models and human AD patients, and that increased SINE RNA processing is associated with Alzheimer's disease.

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#### **P115 Effects of somatostatin binding to A $\beta$ aggregates studied by molecular dynamics simulations and essential collective dynamics analyses**

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Its prevalence in the aging population, difficulties in early diagnosis, and the lack of efficient therapeutics make Alzheimer's disease (AD) one of the most devastating neurodegenerative disorders of our time. AD is associated with the misfolding and formation of toxic aggregates of the amyloid  $\beta$  (A $\beta$ ) peptide [1]. It is believed that small soluble oligomers are particularly toxic. However despite rapidly growing efforts, molecular mechanisms of misfolding and aggregation, as well as co-factors that might activate these processes remain elusive. In particular, toxic A $\beta$ \*56 complexes have been hypothesized to require unknown co-factors for assembly [2].

The cyclic peptide somatostatin-14 (SST14) was recently found to be the most selectively enriched protein in human frontal lobe extracts that binds oligomeric A $\beta$ <sub>1-42</sub> aggregates [3]. Furthermore, SST14's presence was found to inhibit fibrillization of A $\beta$ <sub>1-42</sub> in vitro, while promoting formation of smaller oligomers, reminiscent of A $\beta$ \*56 complexes [3,4]. Interestingly, A $\beta$ <sub>1-42</sub> but not A $\beta$ <sub>1-40</sub> peptides were prone to form such complexes under the influence of SST14.

In order to elucidate the mechanisms of the SST14 interaction with A $\beta$  oligomers, we have prepared several *in silico* models of A $\beta$ <sub>1-42</sub> aggregates, comprising quasi-globular A $\beta$ <sub>1-42</sub> oligomers

and fibrillary constructs 2M4J and 2NAO [5]. We performed all-atom molecular dynamics (MD) simulations of these aggregates in the presence of SST14, and compared structure, dynamics and stability of each system with control SST14-free simulations. For this purpose, we have combined well-established structural analysis tools with a novel essential collective dynamics method [6] developed in our group.

SST14 has shown a strong propensity to interact with small, quasi-globular A $\beta$ <sub>1-42</sub> oligomers, with a tendency of becoming part of existing oligomers, accompanied by a buildup of  $\beta$ -content in SST14. Attachment of SST14 to these oligomers induced changes of their local dynamical stability. In some simulations, SST14-exposed oligomers showed increased stability at specific locations. In contrast, most SST14-free oligomers have retained their dynamical fingerprints largely unchanged. Binding of SST14 to the N-termini of A $\beta$  chains in fibrillary constructs 2M4J and 2NAO has led to local fibril disruption and loss of stability.

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[6] L. Dorosh and M. Stepanova. *Molecular BioSystems*, 13 (2017) 165-182.

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## **P116 Role of the Prion Protein in Synucleinopathies**

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Alpha-synuclein is highly prevalent in neurons and in its pathogenic conformation it is cause for severe synucleinopathies like Parkinson's disease. Typical hallmark are the oligomerisation and aggregation of alpha Synuclein forming fibrillar structures. Several mechanisms have been shown to be involved in the proliferation of fibrillar alpha-Synuclein in the brain: tunnelling, endocytosis and micropinocytosis. Still the mechanism behind the cell-to-cell spreading of alpha-Synuclein is to be established. One hypothesis is that the Prion protein, which is highly expressed in neurons, plays an important role for the alpha-Synuclein uptake as a possible receptor. To support that theory secondary cell cultures with different PrP<sup>C</sup> expressions levels are being compared in their ability to assimilate recombinant alpha-Synuclein. Results show that by deviating amounts of PrP<sup>C</sup> the amount of alpha-Synuclein varies. Additionally, special phosphorylation of interest of alpha-Synuclein (p129) as well as the localization of the added protein is to be investigated within the cells. In summary the binding and absorption of alpha-Synuclein is enhanced by an increased amount of PrP<sup>C</sup> on the surface of the cells. These observations can provide a better understanding of the pathological mechanism of the alpha Synuclein spreading and represents a possible target for therapeutic drug development.

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## **P117 Molecular basis for seeding barriers between three- and four-repeat tau**

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## **P118 Misfolding and Aggregation of Amyloidogenic Proteins in Intra- and Extracellular Environments**

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Structural characterization of amyloid (A) $\beta$  peptides implicated in Alzheimer's disease (AD) is a challenging problem due to their intrinsically disordered nature and their high propensity for aggregation. This structural information is important for understanding molecular mechanisms of pathology as well as for developing and optimizing therapeutic agents for intervention in AD. Despite significant efforts, there is no consensus on conformational properties and aggregation pathways of A $\beta$  peptides in cellular environments. The situation is further complicated by the fact that peptides can aggregate and perform their neurotoxic activity intracellularly. The intracellular A $\beta$  species can also be secreted into the extracellular space where they may trigger prion-like pathological misfolding.

*In silico* modeling can complement biophysical experiments, providing atomistic insight into structure and dynamics of different A $\beta$  species. All-atom explicit solvent molecular dynamics (MD) simulations can deliver reliable structural information. In the case of intrinsically disordered A $\beta$  peptides, extended sampling simulations at the microsecond time scale are required to obtain statistically meaningful results even for simple solvent conditions. To overcome the challenges of conformational sampling in crowded cellular environments, alternative approaches have been developed, including post-processing of MD data. In this study, we employ the statistical-mechanical, 3D-RISM-KH molecular theory of solvation to describe solvent composition effects on the conformational equilibrium between different A $\beta$ 42 species based on the statistical reweighting technique. The methodology enables a computationally efficient prediction of effects of different factors in the cellular environment (such as solvent ionic composition, nonpolar solvation, and macromolecular crowding) on the structural properties of biomolecules.

Similarities have been identified between changes in the structural ensemble of the A $\beta$ 42 monomer caused by nonpolar solvation and crowded environments. In particular, both solvent conditions reduce random coil content and enhance  $\alpha$ -helical structural content. In contrast to previous studies, this work attributes these structural features to the asymmetry in charge distribution of the A $\beta$ 42 monomer as well to the difference in hydration properties of oppositely charged ions, rather than the entropic excluded volume effects.

The methodology developed in this study to describe the effects of environmental factors on structural properties of intrinsically disordered proteins can also be used to predict the potential of anti-aggregation drug candidates to shift the conformational balance away from aggregation-prone states and to map druggable sites (hot spots) of amyloidogenic proteins. This feature is



illustrated by characterization putative druggable sites of A $\beta$  peptides and the Zn/Cu SOD1 enzyme implicated in ALS.

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#### **P118B In-silico design and optimization of small molecules for inhibition of prion-like propagation at Trp32 site of SOD1 enzyme in Amyotrophic Lateral Sclerosis**

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Misfolding of human Cu/Zn superoxide dismutase (SOD1) metalloenzyme results in the formation of neurotoxic oligomers in familial Amyotrophic Lateral Sclerosis (fALS). There is no effective treatment available to prevent the degeneration of motor neurons in ALS patients. The human cell culture studies demonstrated that misfolded G85R-SOD1 propagate from cell to cell in a prion-like fashion and Trp32 residue is important for templated misfolding of wild type-SOD1 (wtSOD1) [Grad et al., PNAS 108: 16398 (2011)] into toxic aggregates. The inhibition of pathological misfolding of SOD1 by small compounds binding at Trp32 is a novel and promising therapeutics strategy for the development of ALS therapy. The stabilization of SOD1 dimer interface is another strategy to prevent misfolding and aggregation. Several drug-like molecules were screened in silico to stabilize the dimer interface of SOD1. However, 5-fluorouridine (5-FUrd), one of the candidate for stabilization of dimer, binds to Trp32 residue in contrary to the originally proposed dimeric interface of SOD1 (Wright et al., Nat. Commun. 4:1758 (2013), PDB code:4A7S). A novel protocol was developed and applied to account for structural solvation effects to predict binding modes of 5-FUrd at Trp32 site. The protocol combines water placement algorithm based on the three-dimensional reference interaction site model (3D-RISM) integral equation molecular theory of solvation complemented with the Kovalenko- Hirata (KH) closure approximation and docking implemented in the Molecular Operating Environment (MOE) integrated drug discovery package. Docking experiments with 5-FUrd revealed that structural solvation plays a crucial role in ligand recognition and binding to the Trp32 binding site. De novo drug design and pharmacophore-restrained high-throughput virtual screening (HTVS) methods were used to identify the anti-ALS lead candidates at Trp32 site. Based on analysis of physico-chemical descriptors and pharmacophore features of the experimental binding mode of 5-FUrd at Trp32, a group of new compounds were rationally designed with de novo drug design methods. A library of commercially available lead-like compounds (650,000) and Food and Drug Administration (FDA) approved small molecules were screened at Trp32 site of SOD1 using HTVS and were filtered through the uracil based pharmacophore model. Lead compounds were selected with virtual screening, and de novo drug design methods show higher affinity and can potentially inhibit prion-like propagation of misfolding of SOD1. Anti-ALS lead candidates were ranked based on molecular descriptors which help to predict the blood brain barrier (BBB) permeation. One of the anti-ALS lead compound (L5) tested in ALS-zebrafish embryo model significantly rescued axonopathy in a dose-dependent manner.

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#### **P119 Verification for overexpression values of plasma protein markers: Applied to sporadic Creutzfeldt-Jakob disease onset prediction**

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Sporadic Creutzfeldt-Jakob disease (CJD) is a human prion disease that rapidly progresses and is fatal and characterized by spongiform encephalopathy, which is abnormally aggregated prion protein (PrP<sup>Sc</sup>), in the brain. Patients with CJD are usually diagnosed according to their clinical manifestations, electroencephalography findings, increased 14-3-3 cerebrospinal fluid levels, and neuroimaging. But the various alternative diagnostic indicator substances being developed owing to 14-3-3 with high false positive results or low specificity.

129M type humanized transgenic mice (KRhuPrP129M) expressing human prion protein (PRNP) were divided into a normal group and infectious group, the brain homogenate of which was inoculated with PrP<sup>Sc</sup>. Proteomic approach, multiple reaction monitoring-mass spectrometry, and immunological technique, immunoblot, were performed on the plasma of five individuals for each day post-infection (dpi). Additionally, their protein levels were quantified using commercial kits.

Of the candidate proteins, four differed significantly between the groups. These proteins, which were strongly upregulated in the transgenic mice compared with the normal mice, exhibited PrP<sup>Sc</sup>-dependent increases. The results of the proteomic analysis and immunological assays including the immunoblot and enzyme-linked immunosorbent assay, were similar.

Because CJD presents features of various neurological disorders, brain biopsies or autopsies are required for definitive diagnoses, but these procedures are controversial in Korea. These verified biomarkers can be used to diagnose patients in each phase. Therefore, these results show that indicator biomarkers can be useful for understanding the underlying pathogenesis of the human prion diseases.

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### **P119B Cross-interaction between $\alpha$ -synuclein and other amyloidogenic proteins: preliminary aggregation and morphological studies**

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$\alpha$ -synuclein is an amyloidogenic protein implicated in different neurodegenerative disorders, including Parkinson disease, Dementia with Lewy Body and Multiple System Atrophy, also known as synucleinopathies. Once misfolded, this protein can acquire different conformational variants (strains) that account for the huge phenotypic heterogeneity associated with these diseases. Interestingly, other than propagating its misfolding in an auto-catalytic manner,  $\alpha$ -syn might induce the conformational conversion of other amyloidogenic proteins, including tau and

amyloid- $\beta$  ( $A\beta$ ). In turn, these proteins can influence  $\alpha$ -syn aggregation. How these proteins interact and influence each other's aggregation is still unknown. We decided to analyze this aspect using the Real Time Quaking Induced Conversion (RT-QuIC) assay, especially for evaluating the effects that different amyloidogenic proteins exert on  $\alpha$ -syn aggregation. As RT-QuIC substrate we used two different batches of  $\alpha$ -syn named  $\alpha$ -SynJ and  $\alpha$ -SynC, produced in two different labs.  $\alpha$ -SynJ was able to self-aggregate (in the presence of water and Thioflavin T) at around 70 hours. This aggregation was accelerated when minute amount of pre-formed  $\alpha$ -SynJ amyloid fibrils (femtograms) were added to the reaction. Surprisingly, the addition of other amyloid fibrils made up of tau, tau fragments (K18 and K19),  $A\beta_{1-40}$  and  $A\beta_{1-42}$  did not accelerate  $\alpha$ -SynJ aggregation kinetic. We are currently producing heterogeneous amyloid fibrils by incubating combinations of different monomeric proteins (e.g. tau+ $A\beta_{1-42}$ ) in order to assess their ability to modify aggregation and structural properties of  $\alpha$ -synJ. We have then analyzed the aggregation properties of  $\alpha$ -SynC (using the same experimental conditions of  $\alpha$ -SynJ) which was able to self-aggregate at around 40 hours. Both  $\alpha$ -SynJ or  $\alpha$ -SynC amyloid fibrils were added to  $\alpha$ -SynJ or  $\alpha$ -SynC substrates and, in both cases,  $\alpha$ -SynC induced a more rapid aggregation of the substrates than  $\alpha$ -SynJ. Biochemical and Transmission Electron Microscopy studies confirmed the presence of biochemical and morphological differences between  $\alpha$ -SynJ and  $\alpha$ -SynC. These data suggest that, even in the absence of specific cofactors, we could generate two conformational variants of  $\alpha$ -syn characterized by different RT-QuIC seeding abilities when assessed on  $\alpha$ -syn or tauK18. These results should be taken into account when RT-QuIC is used for analyzing biological samples collected from patients with synucleinopathies which are indeed likely to contain different strains of  $\alpha$ -syn (and other amyloidogenic proteins) with different seeding abilities (that influence sensitivity and specificity of the test). Our aim is to optimize RT-QuIC for detecting  $\alpha$ -syn in the olfactory mucosa (OM) of patients with different synucleinopathies. Considering that OM might contain  $\alpha$ -syn but other protein aggregates, our cross-seeding studies might be fundamental for understanding the results of such analyses.

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**P120 “Differential interactome of aggregation prone/prion like proteins under stress”: novel links to cellular stress signaling**

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Aims: Misfolding/aggregation of different proteins in specific brain regions is a central event in many neurodegenerative diseases. Emerging evidences are linking protein aggregation to altered cellular stress response. The complete identification of initial makeup of aggregate prone/prion like proteins in disease have been major hurdle to progress in understanding how cells deal with stress, where one measurable outcome of that stress is the misfolding and subsequent aggregation of proteins. In this study, we aim to identify full interactome of aggregate prone proteins (Tau, PrP,  $\alpha/\beta/\gamma$  synuclein) in normal and cells under stress to find out which specific Interactors are recruited to these aggregated Prone proteins upon oxidative stress.

Methods: The human cervical adenocarcinoma (HeLa) cell line was used for interactome mapping of target proteins. For stress induction, cells were incubated at 37 °C for 60 minutes with 0.6 mM sodium arsenite and target proteins were affinity captured along with their interacting partners by their specific antibodies immobilized on protein G magnetic dyna beads, both under normal and stressed conditions. After purification isolated protein complexes were identified comprehensively by LC-MS/MS analysis. The identified interactome was verified by combination of experimental (Immunofluorescence and immunoblotting) and Bioinformatics approaches to explore mechanistic links to disease pathology.

**Results:** Interestingly, we found stress specific alterations in the enriched protein complexes (11 stress specific proteins in Tau-interactome, 14 in PrP and 30 in  $\alpha/\beta/\gamma$  synuclein). Comparative interactome profiling under normal and stress conditions indicated distinct protein clusters that were exclusively recruited to each aggregate prone protein under stress. Selected proteins had functions related to splicing, translation, cytoskeleton and stress response, highlighting mis-splicing and altered protein synthesis machinery under stress. Further we found altered post translational modifications (phosphorylation and acetylation) which are an integral component of the proteotoxic stress response under stress condition. Differential expression analysis of selected target interactors in patient's brain samples and healthy subjects revealed a set of proteins showing significant disease subtype dependent regulation.

**Conclusions:** Our results highlight new players associated with aggregation prone proteins under stress, linking cellular stress axis to pathophysiology of these proteins. Further PTMs and differential expression regulation of selected stress specific interactors might reflect distinct elements of the disease process.

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### **P121 RT-QuIC detection of prion and alpha-synuclein seeds in CSF of patients with rapidly progressive dementia**

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Rapidly progressive neurological disorders (RPND) include a heterogeneous group of both neurodegenerative and non-neurodegenerative disorders evolving over days or months. A major issue is to diagnose treatable conditions. A negative (Real-Time Quaking Induce Conversion) RT-QuIC analysis in the cerebrospinal fluid (CSF) and/or olfactory mucosa (OM) excludes sporadic Creutzfeldt-Jakob (sCJD) diagnosis nearly to 100% of patients. By testing CSF samples from non-CJD with alpha-synuclein RT-QuIC (a-syn RT-QuIC) a diagnosis of Dementia with Lewy Bodies (DLB), Alzheimer Disease/LBD, or cerebellar Multiple Systemic Atrophy (MSA) is definitely provided.

In this study, we included 56 patients with RPND clinically interpreted as probable or possible or suspected CJD. Final diagnosis was obtained in all subjects and included 20 subjects with sporadic

CJD, 7 with LBD, 5 with AD, 4 with vascular encephalopathy, 6 with autoimmune and viral encephalitis, 2 with progressive supranuclear palsy, 2 with anoxic encephalopathy, 2 with brain neoplasms, 1 with AD/LBD, 1 with corticobasal degeneration, 1 with Wernicke encephalopathy, 1 FTD-MND, 1 with pontine myelinolysis, and 1 with MSA-C.

CSF samples were tested in blind using firstly prion IQ-QuIC and then a-syn RT-QuIC. Experimental conditions for a-syn RT-QuIC have been set up using brain tissue from alpha-synucleinopathies (PD, LBD, MSA).

IQ-QuIC detected seeding reactions in CSF from 20 sCJD but not in other samples. Alpha-syn RT-QuIC assay was positive in 9 CSF samples, which corresponded to 7 patients with a definite LBD, 1 with MSA-C and one with AD/LBD and seeding reactions were not observed in other cases.

In patients with RPND, a two steps CSF RT-QuIC assay specifically recognizes patients with sCJD and a-synucleinopathies. Although the number of cases studied is still low, these findings indicate that RT-QuIC is highly helpful in the diagnostic assessment of RPND since provides a definite diagnosis 100% specific and sensitive.

Acknowledgments: this study has been supported in part by CJD Foundation.

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## **P122 Amyloid- $\beta$ strain specific profiling in Alzheimer's disease: functional and clinical signature**

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Amyloid- $\beta$  (A $\beta$ ) pathology is one of the salient features of Alzheimer's disease (AD). Over the last decade the self-seeding capability and relative pathogenicity of various A $\beta$  strains has been an active target of research. However, the distinct clinical involvement of these A $\beta$  species in neurodegeneration is yet to be elucidated. Rapidly progressive Alzheimer's disease (rpAD), often misdiagnosed as a prion disease, follows the same biochemical and clinical course but presents a faster cognitive decline and lower survival times. However, the exact molecular mechanisms that make this variant of AD similar to Creutzfeldt-Jakob disease (CJD) are yet to be understood. For this purpose, we aim to decipher the prion-like structural and functional aspects of A $\beta$  aggregation in rapidly progressive dementias.

In this study, we targeted the differences in A $\beta$  species and their roles in progression of the disease. We used affinity-based proteomic profiling to isolate A $\beta$  and its interacting partners from cortical brain regions of slow and rapidly progressive dementia cases i.e. AD, rpAD and CJD, followed by their identification through classical gel based and Top-Down Mass Spectrometry. Our preliminary findings suggest differential metabolism and interactions of this peptide corresponding to different forms of the disease. Several proteins involved in energy metabolism,

neurotransmission, neuronal development and stress response were also found to interact with different A $\beta$  variants. Although the clinical relevance of these alterations is yet to be established, our study presents a comprehensive approach towards the understanding of different A $\beta$  strains and their role in metabolism in association with binding partners leading to the identification of new biomarkers and therapeutic targets.

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### **P123 Prediction of amyloidogenic proteins interacting with amyloid precursor protein**

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Amyloids are protein aggregates with unusual features, such as high resistance to protease and detergent treatment. Investigations of amyloids are in the top of interest due to increasing incidence of amyloidoses, for instance Alzheimer's disease, Parkinson's disease, type II diabetes, etc. The discovery of co-aggregating amyloidogenic proteins has showcased the existence of a new and understudied type of intermolecular interactions. It is now becoming more and more obvious that such interactions might play an important role in pathogenesis of human and animal amyloidoses. Numerous examples of protein coaggregation associated with different amyloidosis, for instance, coaggregation of following proteins was shown: amyloid-beta and synuclein, amyloid-beta and amylin, amyloid-beta and PrP, etc.

To reveal new proteins that can coaggregate with amyloid beta we performed wide scale bioinformatical screening for amyloidogenic proteins interacting with amyloidosis (APP). We analyzed a set of human proteins, which physically interact with APP according to BioGrid database. The ArchCandy program and IUPred tool were used for prediction of amyloidogenic and disordered regions, respectively. Proteins with overlapping aggregation-prone and unstructured sequences were considered as candidates for new amyloids. Such analysis revealed 289 suitable proteins, implicated in different cellular processes. Noteworthy, using this strategy we identified several well-known amyloids, interacting with amyloid beta: SNCA, TDP-43. This fact demonstrated reliability of our approach. Unfortunately we failed to reveal any enrichment of Gene Ontology terms among identified candidates. Further work will be focused on the verification of amyloidogenic properties of these proteins.

The research was supported by the Russian Science Foundation (17-74-10159).

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### **P124 Stabilization of monomeric polyglutamine proteins by all-D-enantiomeric peptide ligands as therapeutic strategy for polyglutamine-diseases**

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Nine different inheritent diseases are known to be caused by elongated poly-glutamine tracts, including Huntington's disease, Kennedy's disease and DRPLA. The elongated polyQ-tracts cause the afflicted proteins to misfold and aggregate into toxic protein assemblies that are thought to be the cytotoxic agent causing severe neurodegeneration, whereby the mutated protein

determines which neurons degenerate. At present there are only symptomatic treatments available. The treatment strategy we are pursuing is the stabilization of monomeric polyglutamine-proteins by suitable ligands to destroy toxic assemblies and to prevent their formation.

We used mirror image phage display selection to identify all-D-enantiomeric peptides as ligands for monomeric polyQ-proteins. We describe the binding properties of some of the identified ligands to a poly-Q-tract of 46 Qs e.g. by SwitchSENSE and SPR measurements. We also describe the influence of these ligands on the aggregation behavior of the poly-Q-protein.

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**P124B      Stabilization of monomeric  $\alpha$ -synuclein by all-D-enantiomeric peptide ligands as therapeutic strategy for Parkinsons disease**

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Parkinsons disease (PD) is a neurodegenerative disorder selectively affecting dopaminergic neurons. Insoluble inclusions, found in affected neurons of PD patients, have been shown to contain amyloid species of  $\alpha$ -synuclein, which led to the hypothesis of a causal connection between amyloid formation and disease pathology in PD and other synucleinopathies. We aimed for the development of all-D-enantiomeric peptides that destabilize and destroy  $\alpha$ -synuclein aggregates by stabilizing its intrinsically unstructured and monomeric form. To this end mirror-image phage display was performed using a D-enantiomeric full-length  $\alpha$ -synuclein as target. Subsequent screening of the enriched peptide variants by ELISA and bio-layer interferometry led to the identification of the most promising sequences. The molecular binding kinetics as well as the ability to decelerate  $\alpha$ -synuclein aggregation was examined using synthetic D-peptides by surface plasmon resonance and ThT-assay, respectively. When applied to aggregation studies with the L-enantiomeric  $\alpha$ -synuclein, several of the identified D-peptides exhibited the ability to modulate the process of  $\alpha$ -synuclein fibril formation. These findings underline the potential of a targeted PD treatment based on small D-peptide binders inhibiting prion-like aggregation.

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**P125    The role of cellular prion protein in beta-amyloid-induced toxicity**

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An important pathological hallmark of Alzheimer's disease (AD) is the accumulation of beta-amyloid (Ab), a neurotoxic peptide derived from beta and gamma-secretase cleavage of the amyloid precursor protein (APP). Previous studies already indicated an important role of the cellular prion protein (PrPC) as the regulator of Ab-mediated toxicity by acting as a potential receptor. In this project, we intend to elucidate the cellular mechanisms how PrPC mediates the toxicity of Ab in AD. To clarify this issue we had established a bi-transgenic mouse line (5xFADPrnp<sup>-/-</sup>) by crossing PrPC knockout mice with 5xFAD mice, a mouse model with familial Alzheimer's disease mutations in APP and Presenilin genes.

We propose that binding of Ab and PrPC are involved in cellular uptake of Ab. However, the exact mechanism and involvement of further downstream signaling proteins had not been described yet. Intracellular Ab may exert several neurotoxic functions, which results in cellular stress and cell death. Interestingly, we observed the knockdown of PrPC in mice may delay the Ab-induced deficits. 5xFADPrnp<sup>-/-</sup> life expectancy has more than doubled, delayed cognitive and motoric impairment in comparison with 5xFAD mice. Our results suggest the loss of PrPC delays the toxicity of amyloid beta. In conclusion, our data support a role of PrPC as a mediator of A $\beta$  toxicity in AD by promoting early onset of disease.

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#### **P126 Neurodegeneration associated-proteins in human olfactory neurons**

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Olfactory pathway and in particular bulb and the olfactory tract are invariably involved in most of neurodegenerative disorders showing the deposition of the specific disease associated proteins such as beta-amyloid, tau, alpha-synuclein, TDP-43 and prion protein (PrP<sup>c</sup>). In patients with prion disease, we set up a diagnostic test for prion detection using nasal brushing. This procedure allows a gentle collection of olfactory neurons (ON) from a wide surface area of olfactory epithelium without damaging the olfactory function since ONs undergo to an ongoing replacing. We carried out an immunohistochemistry, molecular and biochemical study on olfactory brushings obtained from 40 healthy subjects. Each subject underwent to nasal brushing and collected cells were processed for immunostaining and biochemical analyses. Expression of beta-amyloid, tau and phospho-tau, alpha-synuclein, TDP-43 and PrP<sup>c</sup> in mature and immature neurons were characterized by double immunostaining using neuronal markers such as Tuj-1, Synaptophysin, S100, PGP 9.5, Nestin and the specific tissue olfactory marker protein (OMP) for mature ONs.

This study shows for the first time in humans that mature ONs express beta-amyloid, tau and phospho-tau, alpha-synuclein, TDP- 43 and PrP<sup>c</sup> and this expression is closely related with neuronal maturation. In particular, this protein synthesis seems to start when ONs become mature. We also showed that in normal subjects, these proteins undergo to abnormal post- translational modifications, such as phosphorylation and ubiquitination, which lead to protein aggregation with toxic effects on cell metabolism. These evidences might explain why the olfactory system is early involved in neurodegenerative process.

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#### **P127 Generation of amyloid- $\beta$ dimer conformation-specific antibodies**

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Amyloid beta (A $\beta$ ) plaques, consisting of deposits of fibrillar A $\beta$  peptides, are one of the pathological hallmarks of Alzheimer's disease (AD), the most prevalent neurodegenerative disease in humans. Soluble A $\beta$ -oligomers are the precursors for these A $\beta$  plaques and are capable



to modulate synapse structure and function, and impair memory [1]. Notably, soluble A $\beta$ -dimers have been found to be enriched in AD patients and correlate with the progress of disease. So far, the functional characterization of these dimers was hampered by a kinetic equilibrium of different oligomerization and fibrillization states of A $\beta$ . We were able to stabilize amyloid- $\beta$  dimers by an intermolecular disulphide bridge via introduction of a cysteine into position 8 of the amyloid- $\beta$  domain (A $\beta$ -S8C) *in vitro* and *in vivo* [2].

Our objective was now to generate A $\beta$  dimer conformation specific antibodies that could be invaluable as early biomarker for AD, for therapeutically targeting these toxic A $\beta$  entities and for the functional characterization of A $\beta$  dimers.

In order to raise antibodies against dimeric A $\beta$ , PrP knockout mice were immunized with synthetic oxidized A $\beta$ -S8C dimers and hybridoma cell lines generated by standard protocols. Enzyme-Linked Immuno Sorbent and enzyme-linked immunofiltration assays (ELISA and ELIFA) to the immunogen were used to identify monoclonal antibodies that were highly specific for the A $\beta$ -S8C dimer. Candidate clones were subsequently tested via immunoprecipitation of monomeric or dimeric A $\beta$  from cell culture supernatants and brain homogenates of mice either expressing wild type A $\beta$  monomers or mutant A $\beta$  dimers.

We were able to isolate two monoclonal antibodies that specifically bind to A $\beta$ -S8C dimers. These antibodies are currently further validated with human samples. Results will be reported.

**Keywords:** Alzheimer's disease, amyloid- $\beta$ , dimer, antibody

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#### **P128 A novel M205I mutation in PRNP gene without deposits of PrP<sup>Sc</sup>, associated with vascular, Alzheimer's and argyrophilic grain disease co-pathologies in a Spanish patient with rapidly progressive dementia**

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#### **Introduction**

Human prion diseases are characterized by accumulation of PrP<sup>Sc</sup>, an abnormally folded isoform of the host encoded cellular prion protein PrP.

More than 50 mutations in the *PRNP* coding sequence have been described associating with human genetic prion disease. Additionally, some nonsense mutations in the *PRNP* gene have been found to be associated with clinical Alzheimer's disease.

We describe a clinicopathologic and genetic study of a Spanish patient with rapidly progressive mixed dementia and a novel M205I mutation in the prion protein gene (*PRNP*), without deposit of PrP<sup>Sc</sup> studied by immunohistochemistry and Western blot.

#### Case report

A 79 year-old man, with a history of mild mixed dementia, consulted for gradual temporary disorientation and behavioural disorder. Five months before he was independent for basic activities daily living and social relationship. On admission, he did not present special abnormalities during clinical examination. A Cranial CT scan showed multiple lacunar infarctions in basal ganglia, external capsules and thalamus. MRI and EEG was requested but the patient fell into coma and died three days later.

An autopsy of the brain was performed to rule out a Creutzfeldt-Jakob disease. A blood sample was sent to study the *PRNP* gene. Different samples of fresh brain tissue were frozen at -80°C for subsequent Western blot studies.

The brain weighed 1200 g after formalin fixation. The macroscopic study showed moderate cortical atrophy and atheroma plaques in carotid and basilar arteries with moderated stenosis of the vessels. Coronal sections of the brain showed multiple lacunar microinfarcts in the basal ganglia, thalamus and pons, decreased parenchymal consistency in that areas and atrophy of gray matter.

The neuropathological study, in addition to the lacunar infarcts, showed gliosis, Alzheimer's Disease (AD) Neuropathological Changes evaluated according consensus protocols: A2 B2 C3, and argyrophilic grain disease (AGD), stage IV. There was focal patchy vacuolization in upper cortical layers.

No PrP<sup>Sc</sup> deposits were detected by immunohistochemistry or Western blot studies.

The genetic study of *PRNP* gen showed a novel mutation at codon 205 (M205I) previously undescribed. The polymorphism at codon 129 of the *PRNP* gene was MM.

The theoretical study that predicts the potential pathogenicity of the mutation using the reference protein sequence P04156.1 on the PolyPhen .2 server suggests that this mutation is benign, having a minimum impact on the structure and function of the prion protein, PrP.

#### Discussion

The mutation M205I in *PRNP* gen has not been previously described.

In our case it is associated with vascular, AD neuropathologic change and AGD co-pathologies without deposits of PrP<sup>Sc</sup> and clinical rapidly progressive mixed dementia.

It is important to perform neuropathological and molecular studies in dementia to categorize different clinical and neuropathological phenotypes.

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### **P129 PrP as a receptor of alpha-synuclein in the pancreas of patients with synucleinopathies**

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Neurodegenerative diseases such as Alzheimer's disease or Parkinson's disease are characterized by the progressive dysfunction and death of some nerve cells. Specific protein aggregates constitute the defining neuropathological characteristics of these diseases. One of the key events in the pathogenesis of neurodegenerative diseases is the ability of the amyloidogenic proteins to enter healthy cells and enhance the conversion of the endogenous protein into the aggregation-prone form. In Parkinson's disease,  $\alpha$ -synuclein fibrils deposit inside neurons and can be released and taken up by other cells. This fact could cause the spreading of the pathology and the neurodegeneration.

We studied pancreatic tissue from 39 subjects diagnosed with Parkinson's disease, Lewy body Dementia or incidental Lewy bodies disease, as well as that from 86 neurologically asymptomatic subjects. We studied the pancreatic tissue to assess the accumulation of PrP. Moreover, we performed proximity ligation assays to assess whether this protein interact in the pancreas of these subjects with the  $\alpha$ -synuclein. We designed two specific PLA assays to detect the interaction between PrP and both the C and N terminal regions of the  $\alpha$ -synuclein. Furthermore, we designed a specific PLA assay to detect the interaction between PrP and phosphorylated  $\alpha$ -synuclein. Recently it has been shown that PrP<sup>C</sup> on the cell surface promotes the uptake of different fibrillar forms of  $\alpha$ -synuclein through a direct binding by its N-terminal domain

We found a significant increase in cytoplasmic PrP expression in pancreatic  $\beta$  cells of subjects with synucleinopathies compared with neurologically asymptomatic subjects. Furthermore, we found a PrP/ $\alpha$ -synuclein interaction in patients with phosphorylated  $\alpha$ -synuclein pancreatic inclusions. This interaction also occurred between the PrP and the phosphorylated  $\alpha$ -synuclein.

Our study shows for the first time, histological evidence of the interaction between PrP<sup>C</sup> and  $\alpha$ -synuclein in pancreatic beta-cells of subjects with synucleinopathies. Although further research is needed, our results are in line with previous reports highlighting that the interaction between PrP<sup>C</sup> and  $\alpha$ -synuclein, could lead to  $\alpha$ -synuclein fibrils internalization. This process may have therapeutical applications, that will be further discussed.

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### **P130 Structurally different amyloid- $\beta$ fibrils induce distinct patterns of brain amyloidosis in animal models of Alzheimer's disease**

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The accumulation of the amyloid- $\beta$  (A $\beta$ ) peptide as amyloid fibrils in the brain is associated with Alzheimer's disease (AD) progression. Compelling evidence suggest that the deposition and spreading of A $\beta$  aggregates in the brain of AD patients occurs by a prion-like mechanism. Indeed, misfolded A $\beta$  aggregates share several features with prions, including the existence of different conformational variants or "strains". It has been shown that amyloid fibrils commonly exhibit distinct morphologies as a consequence of variations in the molecular structure of the protofilaments. In the case of A $\beta$ <sub>1-40</sub>, two morphologically distinct fibrils, 2F and 3F, have been

produced *in vitro* and their structure extensively characterized by electron microscopy and solid state NMR. Both, the morphology and the molecular structure of these aggregates can be faithfully self-propagated *in vitro*. We hypothesized that specific A $\beta$  strains in AD are responsible for different patterns of pathological progression including toxicity, tissue and cell tropism, and prion-like rate of propagation of the aggregates. In the present study, the biochemical and biological properties of 2F and 3F fibrils (generated in the laboratory of Dr. Robert Tycko) were characterized *in vitro* and *in vivo*. The differential resistance to proteolysis of these preparations was assessed by using different concentrations of proteinase K. The capacity of these aggregates to seed protein misfolding was assessed *in vitro* using 2F and 3F aggregates at different concentrations. To analyze the *in vivo* prion-like features of these aggregates, we injected them intra-cerebrally into Tg2576 mice (a model of familial AD) which were sacrificed at different time points. Our results show that PK resistance profiles allow easy differentiation between the 2F and 3F A $\beta$  species. *In vitro* propagation assays show different rates of amplification. Interestingly, end-products differentially reacted with the amyloid-specific dye Thioflavin S. *In vivo* propagation using these aggregates resulted in differential outcomes. Animals receiving 2F fibrils exhibited significantly higher amyloid induction, particularly at the level of A $\beta$ <sub>40</sub> when compared to animals injected with 3F. Moreover, 2F injected animals also presented a significant increase in A $\beta$ <sub>42</sub> levels when compared to 3F injected animals, albeit similar to control subjects inoculated with aged Tg2576 brain homogenate. These results were consistent at both 100 and 250 days post injection. Overall, we found that structurally distinct A $\beta$  fibrils differentially induce brain amyloidosis in susceptible animals, thus raising the possibility that specific amyloid morphologies have different pathogenicity affecting the outcome of AD patients.

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**P131 MiniBrain for Prion-like diseases: A novel platform to study prion like diseases using 3D human neuroectodermic organoids derived from iPSCs**

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Recently was described a protocol designed to obtain 3D human cerebral organoids termed “Mini-Brains” (Lancaster *et al.*, Nature 2013), allowing for the study of the roots of neurodegenerative diseases. We are currently exploring the possibilities offered by this approach to model prion-like diseases using isogenic induced pluripotent stem cells (iPSCs) expressing either a wild type or a mutated form of the protein of interest. As a proof of concept, we propose to model a tauopathy, the Frontotemporal Dementia (FTD), a neurodegenerative disease associated with a prion-like mechanism.

Two main aspects of the technique are studied here: (i) The MiniBrain generation protocol was redesigned in order to increase the reproducibility compared to the original protocol (ii) Using healthy patient derived iPSCs, we were able to engineer these cells with an episomal vector to have them express different forms of Tau, coupled to a fluorescent reporter, involved in FTD.

The modification of the MiniBrain generation protocol allowed us to obtain up to an eightfold increase of the conversion rate (from iPSCs to MiniBrain). Isogenic iPSCs were obtained expressing either wild-type Tau or P301S Tau (FTD causing mutation). The organoids exhibited as soon as 1 month after generation a pathological characteristic of the disease with a hyperphosphorylation of Tau.

This novel approach compared to the existing literature allows for a more reproducible generation of MiniBrains *in vitro*. iPSCs lines expressing our protein of interest in the long term allows for both a study in localization and expression levels during the differentiation. Our novel platform

could provide a basis for high content screening assays for the validation of therapeutic compounds before in vivo assay.

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**P132 Aged cattle brain displays Alzheimer's-like pathology that can be propagated in a prion-like manner**

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Amyloid beta (Ab) and hyperphosphorylated tau (ptau) are the proteins undergoing misfolding in Alzheimer's disease (AD). Recent studies have shown that brain homogenates rich in amyloid aggregates are able to seed the misfolding and aggregation of amyloidogenic proteins inducing an earlier onset of the disease in mouse models of AD. This seeding behavior is analogous to the disease transmission by propagation of prion protein misfolding observed in prion diseases. Prion diseases can be transmitted across species by inoculation of the misfolded prion protein from one specie into an appropriate host. For example, material from cattle affected by bovine spongiform encephalopathy can be propagate in humans inducing variant Creutzfeldt-Jakob disease. In this study, we analyzed the presence of AD-related protein aggregates in the brain of old cows and investigated whether these aggregates are capable to induce pathology in animal models of AD. We observed that many of the typical hallmarks detected in human AD brains, including Ab aggregates and tangles, were present in cow brains. When cattle tissue containing Ab aggregates or ptau were intracerebrally inoculated into APP/PS1 or P301S mice, we observed an acceleration of brain misfolded protein deposition and faster cognitive impairment compared to controls. However, when the material was orally inoculated, no effect was observed. These results may contribute to uncover a previously unsuspected etiology surrounding some cases of sporadic AD. However, the early and controversial stage of the field of prion-like transmission in non-prion diseases added to the artificial nature of the animal models utilized for these studies, indicate that extrapolation of the results to humans should not be done without further experiments.

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**P133 Dual mode of action: anti-PrP peptide aptamers inhibit A $\beta$  oligomer-Prion Protein interaction and reduce A $\beta$  production**

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According to the amyloid cascade hypothesis, an imbalance between the production and clearance of amyloid-beta (A $\beta$ ) is causing the development and progression of Alzheimer's disease (AD). A $\beta$  oligomers (A $\beta$ O) are the main toxic species. Toxicity is partly induced by the interaction of A $\beta$ O with cellular prion protein (PrP<sup>C</sup>). Furthermore, PrP<sup>C</sup> is inhibiting the BACE1 enzyme, which catalyzes the rate-limiting step in the production of A $\beta$ . Previously, we selected peptide aptamers (PA) against PrP<sup>C</sup> to inhibit the conformational conversion of PrP<sup>C</sup> to the infectious

PrP<sup>Sc</sup> in prion diseases. The used PAs consist of a 16 amino acid peptide motif integrated into bacterial thioredoxin A (trxA) as a scaffold protein. One PA, termed PA8, binds at aa 100-120 to PrP<sup>C</sup>, which is the A $\beta$ O-PrP<sup>C</sup>-binding site. Additionally, we optimized this PA by single amino acid substitutions affecting its binding affinity to PrP<sup>C</sup>. PA8 and its derivatives, termed 46K, 46Q, and 47H, are increasing the expression of PrP<sup>C</sup> at the cell surface and the  $\alpha$ -cleavage of PrP<sup>C</sup> into an N1 and C1 fragment.

In this study, we evaluate the potential of PA8, 46K, 46Q and 47H as therapeutic drugs for AD. Our primary goal was to inhibit the A $\beta$ O-PrP<sup>C</sup>-interaction and consequently reduce neuronal toxicity, inhibition of long-term potentiation, and ultimately memory impairment in AD mice. Treating wtN2A cells with A $\beta$ O led to a reduced cell viability of  $34 \pm 7\%$ , detected by MTT assay. The additional treatment of wtN2A cells with PA8 and 46K for 24 h resulted in a significant increase of cell viability to  $44 \pm 8\%$  and  $48 \pm 16\%$ , respectively. Treating SH-SY5Y cells with PAs, followed by MTT assay confirmed that the enhanced cell viability is PrP<sup>C</sup> dependent, because this cell line does not express PrP<sup>C</sup>. Surprisingly, extra- and intracellular A $\beta$  levels are significantly reduced after treating N2A APPswe cells for 3 days. By using an ELISA to quantify A $\beta$  levels, we demonstrated that 46K and 46Q reduced the extracellular A $\beta$  levels significantly to  $32 \pm 19\%$  and to  $40 \pm 18\%$ , respectively. This implicates that PA treatment inhibits A $\beta$  production. Immunoblot detection of the sAPP $\alpha$  fragment showed that the non-amyloid pathway is not significantly impaired. Treating N2A APPwt cells with the PAs did not change the extracellular and intracellular A $\beta$  levels. We hypothesize that the increased PrP<sup>C</sup> level on the cell surface after PA treatment has an inhibiting effect on BACE1. To further investigate the mechanism behind the reduced A $\beta$  production, mass spectrometry will be used for the quantification of different proteins. Furthermore, in vivo studies, treating 5x FAD mice intra ventricularly with osmotic Alzet<sup>®</sup> pumps are in preparation.

In conclusion, the here used PA PA8, 46K, 46Q, and 47H were able to inhibit the A $\beta$ O-induced toxicity and reduce A $\beta$  production in vitro. These findings indicate that PAs are interesting candidates for the treatment of both prion diseases and AD.

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### **P134 $\alpha$ -Synuclein Strains Propagate Via Prion-Like Templating**

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A prion-like mechanism has been proposed to explain the apparent spreading of  $\alpha$ -synuclein aggregates in the brains of Parkinson's disease patients. A key feature of prions is that they can exist as distinct strains, and previous work has revealed that  $\alpha$ -synuclein aggregates can exhibit strain-like behaviour. However, whether prion-like conformational templating occurs when  $\alpha$ -synuclein aggregate strains are propagated *in vivo* remains unclear. Here, we demonstrate that inoculation of transgenic mice with different strains of recombinant  $\alpha$ -synuclein fibrils produces clinically and pathologically distinct synucleinopathies. Inoculation with low stability fibrils resulted in short disease incubation periods, granular  $\alpha$ -synuclein deposits, and the induction of cerebral  $\alpha$ -synuclein aggregates with low stability. In contrast, injection with high stability fibrils caused prolonged disease incubation periods, Lewy body-like aggregates, and the production of  $\alpha$ -synuclein aggregates with high stability. These differences were maintained upon serial passaging in transgenic mice, suggesting that  $\alpha$ -synuclein strains propagate via prion-like

templating. Our results reveal that  $\alpha$ -synuclein strains exhibit all the biochemical and pathological hallmarks of prion strains. This suggests that prion-like propagation, as opposed to selective neuronal vulnerability, is the dominant mechanism driving the observed spread of  $\alpha$ -synuclein aggregates in Parkinson's disease and related illnesses.

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### **P135 Unravelling the complexity of human neurodegenerative diseases within a bacterial-inspired framework**

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Neurodegeneration is linked to cytotoxic protein amyloid aggregation, as clearly inferred from animal and cell culture models. Protein amyloids arise from the conformational conversion and templated assembly of an otherwise soluble protein into fibrillar aggregates with a crossed  $\beta$ -sheet backbone [1,2].

We have taken the challenge of enabling in bacteria, through Synthetic Biology, a minimal model system providing insight on the molecular basis of intracellular protein amyloidosis [3]. In bacteria, amyloids assemble as functional extracellular scaffolds but no natural proteinopathic amyloidosis has been found in microorganisms yet. However, the bacterial protein RepA-WH1 can assemble into amyloid fibres upon binding to plasmid-specific DNA sequences [4]. RepA-WH1 causes in *E.coli* an amyloid proteinopathy [5,6] which is *vertically* transmissible but not infectious, enabling conformational templating by cross-seeding *in vitro* and *in vivo* [7].

In order to establish on a firm basis RepA-WH1 as a model for amyloid diseases and to ascertain whether the toxicity outlined in *E.coli* [6] can be extrapolated to the much more complex mammalian cells, it is mandatory to express RepA-WH1 in a mammalian cells line and then to dissect the pathways of disease in this host.

We have recently found that transient expression of RepA-WH1 in mouse neuroblastoma N2A cells results in cell death [8].

We are also testing the ability of RepA-WH1 to cross-seed amyloidogenesis on the soluble (WT) variant of the prionoid. For this purpose, cell-to-cell transmissibility experiments are addressed by co-cultivation of both naïve receptor cells and donor cells suffering from RepA-WH1 amyloidosis, as shown before for the yeast prion Sup35-NM in human cells [9]. Indeed, we further study the uptake by cultured cells of pre-assembled amyloid RepA-WH1(A31V) fibres labelled with Alexa 647.

If cross-aggregation of the soluble cytoplasmic protein on the internalized amyloid particles takes place, this would parallel the observation made for Tau,  $\alpha$ -synuclein or SOD-1, showing that RepA-WH1 prionoid indeed recapitulates in human cells a prion-like phenotype [10].

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[2] Structure 18: 1244-1260 (2010).

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- [4] Proc Natl Acad Sci U S A 104: 17388-93 (2007).
- [5] Mol Microbiol 77: 1456-69 (2010).
- [6] Front Microbiol 8: 539 (2017).
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- [8] Prot Cell 5: 737-749 (2014).
- [9] Proc Natl Acad Sci USA 110: 5951-5956 (2013).
- [10] Nature 501: 45-51 (2013).

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### **P136 Analysis of A $\beta$ aggregates using conformation-sensitive dyes**

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The amyloid cascade hypothesis posits that the aggregation and deposition of the  $\beta$ -amyloid (A $\beta$ ) peptide in the brain is the initial step in the pathogenesis of Alzheimer's disease (AD). A growing body of evidence strongly suggests that A $\beta$  aggregates might exist in different conformations with distinct biochemical properties, similar to the existence of prion strains in prion disease. The presence of different conformations of A $\beta$  aggregates might explain the diversity of clinical phenotypes observed in AD. Recently, conformation-sensitive dyes such as luminescent conjugated oligothiophenes (LCOs) and curcumin have been shown capable of differentiating between A $\beta$  aggregates. Differences in the fluorescence emission spectra obtained following binding of these dyes to A $\beta$  aggregates in tissue samples are believed to indicate unique three-dimensional structures. We have developed a fast and reliable microplate-based method for assessing differences in the fluorescence emission spectra of A $\beta$  aggregates in solution following staining with conformation-sensitive dyes. We tested this protocol with a variety of dyes such as LCOs, curcumin, and Thioflavin T and have obtained highly reproducible results. Using this method, we compared the spectral properties of A $\beta$  aggregates composed of either synthetic wild-type (WT) A $\beta$  or mutant A $\beta$  carrying the Arctic mutation (E22G). We found significant differences in the spectral characteristics of A $\beta$  aggregates containing Arctic-mutant A $\beta$ . In addition, synthetic WT and Arctic-mutant A $\beta$  aggregates behaved differently when subjected to a conformational stability assay. Taken together our results suggest that Arctic-mutant A $\beta$  adopts a unique conformation when aggregated, which may explain the strain-like behavior of A $\beta$  aggregates from Arctic AD patients observed upon propagation in transgenic mice. The method described here may also be useful for rapidly discerning conformational variation in aggregates composed of other disease-associated proteins such as  $\alpha$ -synuclein and PrP.

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### **P137 Proteasomal stress induces nuclear localization of the PrP-like Shadoo protein**



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The *Sprn* gene exhibits coding sequence homology to the hydrophobic region of PrP<sup>C</sup>. Its protein product, Shadoo (Sho), like PrP<sup>C</sup>, is neuronally-expressed and has a propensity to aggregate and acquire protease resistance *in vitro*. Sho and PrP<sup>C</sup> differ in their configuration of N-terminal natively disordered sequences - Sho contains RGG repeats while PrP<sup>C</sup> contains PHGGGWGQ metal-binding octarepeats - but both proteins are imported into the ER, modified with N-linked glycans and directed to lipid rafts by virtue of GPI-anchors. In the case of Sho, diversion from the secretory pathways to mitochondria has been reported under conditions of ER stress. Remarkably, as prion-infected brains gradually accumulate PrP<sup>Sc</sup>, there is a converse reduction in the levels of glycosylated forms of endogenous Sho and PrP<sup>C</sup>; moreover, this reduction occurs as a preclinical event. To begin to address whether concerted alterations in proteolytic system might affect the expression of Sho and PrP<sup>C</sup>, we tested three modulators (MG132, NH<sub>4</sub>Cl, and 3MA) that target proteostatic networks.

In primary mixed neuronal and glial cells culture (MNGC) derived from Tg.*Sprn* transgenic mice expressing Sho from a PrP gene promoter, an MG132-mediated reduction in proteasomal activity was associated shifts the repertoire of Sho species towards non-glycosylated forms; these data indicate impaired transport into the ER. Conversely, two autophagic modulators (NH<sub>4</sub>Cl and 3MA) affected neither the expression of Sho or PrP<sup>C</sup>. *In vitro* removal of N-linked glycans by PNGaseF treatment revealed that PrP<sup>C</sup> and amyloid precursor protein (APP) continued to undergo a maturation process through ER under MG132-induced proteasomal stress condition, whereas Sho did not. We also examined murine N2a neuroblastoma cells with Sho expression directed by a housekeeping gene (human elongation factor 1 $\alpha$ ) promoter; here proteasomal stress conditions produced a shift from glycosylated to non-glycosylated forms similar to that seen in MNGC cultures. In contrast to other experimental paradigms, some of which use cytomegalovirus promoter elements to control Sho mRNA expression, neither MNGCs nor N2a cells showed a net increase in Sho under conditions of cellular stress. As determined by immunocytochemistry and western blot analysis of subcellular fractions, application of MG132 to N2a cells resulted in a redirection from intracellular compartments of the secretory pathway and the cell-surface towards accumulation in nuclei. These latter findings reconcile with earlier data detecting low amounts of nuclear Sho under basal conditions using immunohistochemistry or using fluorescent fusion proteins and also align with known *in vitro* nucleic acid binding properties of Sho's low complexity N-terminal domain. While it remains to be established whether re-localization of Sho is associated with reduced half-life or elicitation of an adaptive cellular response, our data reveal Sho location as a sensitive indicator of proteasomal stress.

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### **P138 Cytosolic prions derived from a glutamine/asparagine-rich yeast prion domain sequester multiple components of ribonucleoprotein complexes in mammalian cells**

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Approximately one percent of the mammalian proteome contains low complexity domains compositionally similar to the domains that drive the formation of infectious protein aggregates in lower eukaryotes. While prion proteins of *Saccharomyces cerevisiae* spontaneously form stable amyloids that propagate over multiple cell generations, so-far studied mammalian proteins with comparable low complexity prion-like domains mediate the formation of transient membraneless multiprotein granules. Familial mutations in low complexity domains of granule proteins result in cytosolic inclusions, suggesting that stress granules could evolve into stable protein aggregates. The molecular events that enable weak multivalent interactions among proteins with low complexity domains or stable aggregate formation remain unclear. Here we demonstrate that the prototype yeast prion domain Sup35 NM that can form stable prion aggregates in mammalian cells is also recruited to transient stress granules. Importantly, recruitment to either prion aggregates or stress granules is most efficient when the carboxyterminal region of the N domain is present. Mass spectrometry analysis reveals that Sup35 NM prions sequester multiple RNA-binding proteins and proteins that are components of RNA granules. The fact that dynamic stress granules and stable self-perpetuating prion aggregates sequester similar interactors supports the hypothesis that stress granules might serve as nucleation sites for the formation of stable aggregates.

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### **P139 A novel small molecule inhibits $\alpha$ -synuclein aggregation, disrupts amyloid fibrils and prevents degeneration of dopaminergic neurons**

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Parkinson's disease (PD) is characterised by a progressive loss of dopaminergic neurons, a process that current therapeutic approaches cannot prevent. In PD, the typical pathological hallmark is the accumulation of intracellular protein inclusions, known as Lewy bodies and Lewy neurites, which are mainly composed of  $\alpha$ -synuclein. Here, we exploited a high-throughput screening methodology to identify a small molecule able to inhibit  $\alpha$ -synuclein aggregation. This molecule, named SynuClean-D, significantly reduces the *in vitro* aggregation of *wild-type*  $\alpha$ -synuclein and the familiar A30P and H50Q variants in a substoichiometric molar ratio. This compound prevents fibril propagation in protein misfolding cyclic amplification assays, and decreases the number of intracellular inclusions in a cell-based model. Computational analysis suggests that SynuClean-D can bind to cavities in mature  $\alpha$ -synuclein fibrils and, indeed, it

displays a strong fibril disaggregation activity. The treatment with SynuClean-D of two Parkinson's Disease *Caenorhabditis elegans* models, expressing  $\alpha$ -synuclein either in muscle or in dopaminergic neurons, significantly reduced the toxicity exerted by  $\alpha$ -synuclein. SynuClean-D treated worms showed decreased  $\alpha$ -synuclein aggregation in muscle and a concomitant motility recovery. More importantly, this compound is able to rescue dopaminergic neurons from  $\alpha$ -synuclein induced degeneration. Overall, the data we have obtained validated our screening method as a valuable tool for searching compounds that, as SynuClean-D, have the potential for therapeutic intervention in PD.

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**P140 CNS-derived extracellular vesicles from human mutant SOD1 transgenic mice possess misfolded SOD1 and transmit SOD1 prion-like propagated misfolding**

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Extracellular vesicles (EVs) are known to participate in the propagation of prion infection in cultured cells, and we have previously shown that cell line-derived exosomes possess misfolded SOD1 that may participate in the prion-like propagated misfolding of this protein in amyotrophic lateral sclerosis (ALS; Grad et al, PNAS 2014). We developed a protocol to collect EVs from frozen whole murine neural tissues by serial centrifugation and purification on a sucrose cushion. EVs from SOD1<sup>G93A</sup> transgenic ALS mouse brains and spinal cords possess abundant misfolded SOD1, lumen >> surface. Quantitative proteomics found that brain-derived EVs contain canonical exosomal markers, such as the normal cellular isoform of the prion protein. The brain EVs contained numerous proteins implicated in ALS such as RNA binding proteins, and SOD1<sup>G93A</sup> transgenic EVs were significantly depleted in myelin-oligodendrocyte glycoprotein compared to non-transgenic animals, consistent with reports of oligodendrocyte dysfunction in human ALS and SOD1<sup>G93A</sup> mice (Philips et al, Brain 2013). Brain and spinal cord EVs were positive for the astrocyte marker GLAST and the synaptic marker SNAP25, while CD11b, a microglial marker, was largely absent, suggesting that microglia do not contribute to the tissue EV population under these conditions. For transmission studies, we exploited the fact that the natural familial ALS SOD1 frameshift/deletion mutant G127X lacks an epitope at the C-terminus of misfolded full-length SOD1 recognized by the misfolding-specific antibody 3H1 (Grad et al PNAS 2011; Pokrishevsky et al Sci Rep 2016). CNS-derived EVs from SOD1<sup>G127X</sup> mice were potent inducers of human wild-type SOD1 (HuWtSOD1) misfolding in primary spinal cord mixed neuron-glia cultures derived from HuWtSOD1 transgenic mice, which displayed 3H1 immunoreactivity in morphologically identifiable neurons and astrocytes. Analysis of SOD1<sup>G127X</sup> CNS-EVs revealed high molecular weight G127X multimers that collapsed to monomers upon disulfide reduction, suggesting that the SOD1 prion-like seeding species may constitute G127X aggregates stabilized by non-native disulfide bonds. Our data demonstrate that CNS-EVs derived from mutant SOD1 disease-affected mice contain a seeding template for prion-like propagated misfolding of SOD1, and that astrocytes and neurons are the main source and recipient of these seeding CNS-EVs.

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**P141 Propagation of different human  $\alpha$ -synucleinopathies in mouse model**

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Synucleinopathies are neurodegenerative diseases characterised by the abnormal accumulation of aggregates of  $\alpha$ -synuclein protein in neurons, nerve fibres or glial cells. Recent research supports a prion-like mechanism for the propagation of aggregated  $\alpha$ -synuclein within the nervous system. In case of prion diseases, the existence of different strains that propagate infectivity stably explain their phenotypic heterogeneity. The aim of this work is assessing if the strain phenomenon accounts for differences in disease progression and pathological features in the brain of different individuals suffering synucleinopathies.

Five different groups of brain tissue coming from donor patients showing clinically and histopathologically different synucleinopathies were selected to characterize plausible  $\alpha$ -synuclein strains, and were inoculated in transgenic PAC-Tg (SNCAWT) mice expressing human  $\alpha$ -synuclein in a background null for murine  $\alpha$ -synuclein. To increase the chance to distinguish  $\alpha$ -synuclein strains by differential in-brain propagation, human diseased and healthy human control brains were assessed in three set of experiments using two different inoculation points and two different concentrations of the inocula. During necropsy, brain was collected from each animal and fixed in 10% buffered formalin for histopathological analysis. Immunohistochemical analysis was performed using the anti- $\alpha$ -synuclein phosphorylated (Ser129) 81A antibody.

Although some of these experiments are still ongoing, the preliminary results obtained up to now showed that most of the brains from mice injected at the right parietal lobe or at the left striatum with human diseased brains homogenized at 10% (w/v) in PBS did not show any staining for phosphorylated  $\alpha$ -synuclein up to 21 months post inoculation. On the other hand, some of the brains of mice injected with human diseased brains homogenates five times more concentrated than the previous ones presented a staining for phosphorylated  $\alpha$ -synuclein at 9 months post inoculation. This staining appeared as neurites and also as an accumulation of small abnormal intraneuronal inclusions bodies, spreading from the point of inoculation, the left striatum, caudally to the hippocampus and the cerebral cortex at that level. These preliminary results show that, although at this moment differences between the different inocula cannot be demonstrated, it is possible to conclude that the inoculum concentration induce a considerable reduction in the time to onset of  $\alpha$ -synuclein aggregates appearance.

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## **P142 Misfolding in A $\beta$ and $\alpha$ -synuclein Aggregates Probed by Molecular Modeling**

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Improper folding of proteins followed by formation of toxic misfolded aggregates is associated with a growing group of disorders known as protein misfolding diseases. Despite their broad variety, these diseases share a molecular mechanism that involves a cascade of self-replicating misfolding events. This is accompanied by formation of oligomeric aggregates, subsequently resulting in deposition of  $\beta$ -rich amyloid fibrils. Toxicity is attributed primarily to small and still soluble oligomers rather than mature fibrils; however it is unclear how exactly the misfolding

occurs, and what is the structure and molecular action of toxic oligomers. A detailed understanding of the nature, structure, dynamics, and reactivity of misfolded oligomers is required for efficient therapies to be developed.

In an effort to better understand molecular details of misfolding and aggregation in disease related proteins, we have investigated early stages of this process in A $\beta$  peptide associated with Alzheimer's disease, and  $\alpha$ -synuclein implicated into Parkinson's disease. Employing all-atom molecular dynamics simulations, we have studied detailed mechanisms responsible for misfolding and oligomerization of these proteins under conditions of molecular crowding. Secondary structure content, hydrogen bonding, solvent accessible surfaces, and other descriptors have been examined. In order to analyze dynamics of predicted aggregates in a greater depth, we have applied novel essential collective dynamics (ECD) method [1-4] developed in our group. We report outcomes of extensive comparative analysis of structural and dynamical changes occurring in A $\beta$ <sub>17-42</sub>, A $\beta$ <sub>1-40</sub>, A $\beta$ <sub>1-42</sub> and  $\alpha$ -synuclein multimeric constructs in bulk water, as well as near water surfaces, at physiologically relevant temperature conditions.

Formation of stable  $\beta$ -enriched aggregates has been observed in most A $\beta$  and  $\alpha$ -synuclein model systems. Minimizing exposures of hydrophobic residues to water appears to be a primary driving force behind formation and stability of these oligomers. Predicted oligomers tend to adopt quasi-globular, largely unstructured conformations with significant presence of random coils and loops. However, ECD analyses have consistently revealed strongly correlated  $\beta$ -rich sub-aggregates in central parts of A $\beta$  oligomers, and regions of synchronized dynamics in  $\alpha$ -synuclein multimeric systems, which may be hypothetically attributed to early stages of proto-fibril nucleation. Metal ions have been found to influence the dynamics and morphology of A $\beta$  oligomers significantly. Proximity to water surfaces appears to facilitate oligomerization.

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### **P143 Whole exome sequencing analyses of possible CJD patients in Korea**

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In this study, 40 DNA samples were purified from possible CJD patients in Korea for the whole exome sequencing and their in-depth analyses. Complex genetic screening analyses were performed on genes, associated with neurodegenerative disease, including Alzheimer's disease, Parkinson's disease and frontotemporal dementia. Rare and possible damaging variants were filtered, and analyzed with pathway analysis tools, such as ClueGo or STRING. Six PRNP variants of 4 pathogenic, 1 common and 1 possibly novel variants were identified. The majority of possible CJD patients did not reveal strong genetic correlations with PRNP, APP, PSEN1 and PSEN2 genes. Instead significant numbers of variants were identified from the genes, which were previously indicated as risk factors in other neurodegenerative diseases. Different possible risk

mutations were found in TREM2 and ABCA7, LRRK2 or SORL1 genes, which were reported to have association with Alzheimer's disease (AD) and Parkinson's Disease (PD). Such involvements of risk genes of AD and PD could suggest few common disease pathways among the various neurodegenerative diseases. Further studies will be carried out to assess their involvement in CJD and its disease progression.

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**P144 Retinopathy in a mouse model of Parkinson's disease: Seeding of synucleinopathy induces accumulation of phosphorylated  $\alpha$ -synuclein (pSer129) and tau, neuroinflammation, metabolic dysregulation, and cell death**

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by accumulation of misfolded  $\alpha$ -synuclein within the central nervous system. Visual problems in PD patients are common, although retinal pathology associated with PD is not well understood. The purpose of this study was to investigate retinal pathology in a transgenic mouse model (TgM83) expressing the human A53T  $\alpha$ -synuclein mutation, and assess the effect of  $\alpha$ -synuclein "seeding" on the development of retinal pathology. Two-month-old TgM83 mice were intracerebrally inoculated with brain homogenate from old, clinically ill (12-18 months) TgM83 mice. Retinas were then analyzed at 5 months of age. We analyzed retinas from 5-month-old and 8-month-old uninoculated healthy TgM83 mice, and old (12-18 months) mice that were euthanized following the development of clinical signs. Retinas of B6C3H mice (genetic background of the TgM83 mouse) served as control. We used immunohistochemistry and western blot analysis to detect accumulation of  $\alpha$ -synuclein, pTau<sup>Thr231</sup>, inflammation, changes in macroautophagy, and cell death. Raman spectroscopy was used to test the potential to differentiate between retinal tissues of healthy mice and diseased mice. This work demonstrates retinal changes associated with the A53T mutation. Retinas of non-inoculated TgM83 mice had accumulation of  $\alpha$ -synuclein, "pre-tangle" tau, activation of retinal glial cells, and photoreceptor cell loss by 8 months of age. The development of these changes is accelerated by inoculation with brain homogenate from clinically ill TgM83 mice. Compared to non-inoculated 5-month-old TgM83 mice, retinas of inoculated 5-month-old mice had increased accumulation of  $\alpha$ -synuclein (pSer129) and pTau<sup>Thr231</sup> proteins, upregulated microglial activation, and dysregulated macroautophagy. Raman spectroscopic analysis was able to discriminate between healthy and diseased mice. This study describes retinal pathology resulting from the A53T mutation. We show that seeding with brain homogenates from old TgM83 mice accelerates retinal pathology. We demonstrate that Raman spectroscopy can be used to accurately identify a diseased retina based on its biochemical profile, and that  $\alpha$ -synuclein accumulation may contribute to accumulation of pTau<sup>Thr231</sup> proteins, neuroinflammation, metabolic dysregulation, and photoreceptor cell death. Our work provides insight into retinal

changes associated with Parkinson's disease, and may contribute to a better understanding of visual symptoms experienced by patients.

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#### **P145 Is Multiple Sclerosis a Transmissible Protein Misfolding Disorder?**

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After years of investigation, the cause of multiple sclerosis (MS) is still not known. Two competing theories propose that on the one hand, MS is driven by a dysregulation of the peripheral immune system promoting inflammatory attacks on the CNS, while on the other hand, an initial primary degenerative process may secondarily trigger autoimmunity. We tested the hypothesis that MS, like many neurodegenerative diseases, is a protein misfolding disorder driven by accumulation of pathological cytotoxic aggregates that can spread through the CSF and CNS. One defining property of such diseases is the transmission of pathology to susceptible hosts. We injected 36 human prion protein over-expressor mice intracerebrally with brain homogenate from 10 different primary or secondary-progressive MS patients. Mice were imaged by 9.4T MRI, then assessed by water maze testing and histology. After 6-12 months incubation, mice injected with MS brain developed significant degrees of MS-like pathology together with cognitive disability, whereas mice injected with control human brain (N = 23 mice from 6 different donor brains) did not develop abnormalities. Passaging (brain homogenate from MS-affected mice injected into a 2nd generation of naïve transgenics) continued to transmit pathology (cont: n=17, MS: n=15). MS-injected mouse brains exhibited significant degeneration of myelin with microglial activation in the corpus callosum, leukocortical junction and peri-ventricular regions, assessed by QD9 and Iba-1 immunohistochemistry. Formic acid-resistant prion protein aggregates were detected in both white and gray matter regions. Protease resistance, often seen in more conventional prionopathies, was not detected in either human MS brain nor MS-inoculated mice. Interestingly, pronounced pathological PrP deposition in both white and grey matter was also observed in the dorsal aspects of lumbar spinal cords after intraperitoneal human MS spinal cord homogenate injection (cont: n=6, MS: n=8). This suggests that pathological prion transmission can also be induced by peripheral inoculation outside the CNS, possibly involving the lymphoreticular and peripheral sensory and autonomic nervous systems like other prion diseases.

**Conclusion:** Our results are consistent with the hypothesis that MS may be a primary degenerative disorder caused by accumulation and propagation of atypical pathogenic prion protein, that can transmit pathology to humanized murine hosts. We speculate that these toxic pathological conformers may circulate in the CSF and spread to various brain regions. The resulting degeneration of myelin and release of antigenic debris could secondarily trigger an autoimmune inflammatory response in genetically and environmentally predisposed hosts. Together, such a convolution of a primary prion-dependent degeneration, with secondary inflammation could explain the broad spectrum of human MS phenotypes.

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#### **P146 Ultrasensitive detection and discrimination of tau seeding activities associated with Alzheimer disease, progressive supranuclear palsy, and other tauopathies**

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Several neurodegenerative diseases are characterized by the presence of intracellular inclusion formed by the aggregation of distinct types of filaments made of hyperphosphorylated tau. Using apparent seeded polymerization mechanisms, tau aggregates with different conformations, or combinations thereof, can propagate consistently in cellular or animal models causing distinct tau pathologies. Accordingly, we have sought practical cell-free methods for sensitively detecting and discriminating various disease-associated tau seeding activities to facilitate studies of tauopathy pathogenesis, and improve diagnostics, prognostics, and therapeutics development. Using the tau RT-QuIC seed amplification platform that we initially optimized for Pick disease, we developed 3 new ultrasensitive assays for other tauopathies such as Alzheimer disease, progressive supranuclear palsy, and corticobasal degeneration. Each new assay is able to detect seeding activity in brain dilutions as extreme as 108-1010-fold in the tauopathy type for which it was optimized, but is orders of magnitude less sensitive in detecting tau seeding activity in other types of tauopathy, despite the presence of similar overall levels of aggregated tau. These results quantitate profound tau seeding selectivities that appear to be associated with different tauopathy types. Such selectivities may help to explain the consistent propagation of distinct variants of pathological tau aggregates in each neurodegenerative disease.

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#### **P146B Cerebrospinal $\alpha$ -synuclein RT-QuIC analysis: a potential diagnostic test for Parkinson's disease**

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A real-time quaking induced conversion (RT-QuIC) assay which detects abnormal CSF  $\alpha$ -syn in Parkinson's disease (PD) was found to have a sensitivity of 95% in an initial study of 20 PD patients.<sup>1</sup> We now report the results of a larger study of CSF samples from 54 PD patients (29M:25F; mean  $\pm$  SD age: 67.1  $\pm$  6.3 years, range: 56.0-82.3) and 54 control subjects (23M:31F; mean  $\pm$  SD age: 65.3  $\pm$  6.9 years, range: 55.1-84.6) from the Michael J Fox Foundation BioFIND cohort. The sensitivity of  $\alpha$ -syn RT-QuIC was found to be 100% with a specificity of 89%. There was no correlation between the  $\alpha$ -syn RT-QuIC positivity and disease severity, age at time of lumbar puncture and cognitive function in the PD patients, however a possible relationship with disease duration was seen. These results are encouraging and a larger prospective study is underway.

#### Reference

Fairfoul G, McGuire LI, Pal S *et al.*, Alpha-synuclein RT-QuIC in the CSF of patients with alpha-synucleinopathies. *Annals of Clin Trans Neurol.* 2016 3(10):812-8

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## IV. Biology of prion precursor proteins

### **P147 Redox regulation of adult neurogenesis by PrP and PrP N-terminal cleavage fragments**

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See O15

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### **P148 Cellular prion protein expression, processing and localization is affected during differentiation of mouse neuronal cell line CAD5**

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Cellular prion protein PrP<sup>C</sup> is considered to participate in various physiological processes, including influencing of cell proliferation and differentiation. To study the role of PrP<sup>C</sup> during differentiation, murine catecholaminergic cell line CAD5 was employed based on its ability to propagate prion infection and to undergo *in vitro* stimulation to differentiation using several approaches. Furthermore, the CRISPR/Cas9 genome editing technology was used to establish control CAD5KO cell line with eliminated PrP<sup>C</sup> expression; the absence of PrP<sup>C</sup> at the protein level was confirmed by Western blot and flow cytometry. The differentiation of both CAD5 and CAD5KO cells was induced by media change accompanied by serum withdrawal and cells were then maintained as a stable culture for up to two weeks. The majority of cells stopped dividing, started creating dendrites and looked morphologically completely differentiated from the 5<sup>th</sup> day after the induction of differentiation, resembling bipolar neurons. However, the quantitative evaluation of cell morphotypes did not show any difference between CAD5 and CAD5KO cells; neither did the cell cycle analysis measured by flow cytometry and the growth analysis of proliferating and differentiating CAD5/CAD5KO cells assessed by the iCELLigence system, even though the expression of PrP<sup>C</sup> in CAD5 cells increased 4–6 times during the tested period of differentiation. Interestingly, the Western blotting data also revealed that PrP<sup>C</sup> undergoes different processing in differentiated CAD5 cells compared to their proliferating counterparts, probably caused by extended  $\beta$ -cleavage of PrP<sup>C</sup> during differentiation. Using flotation in sucrose gradient, lipid rafts were isolated from CAD5 cells after solubilizing membranes with different detergents. After the treatment of cell lysates with Brij 98, larger proportion of PrP<sup>C</sup> was present in lipid raft fractions compared to Triton X-100; nevertheless, both procedures demonstrated that PrP<sup>C</sup> in proliferating cells was almost exclusively raft-associated, whereas a considerable amount of PrP<sup>C</sup> in differentiating cells was found also in non-raft compartments. Although the experiments did not show a major difference in fundamental characteristics of the differentiation process between CAD5 and CAD5KO cells, the analysis of PrP<sup>C</sup> expression in CAD5 cells during

the course of differentiation led to the finding that significantly increased PrP<sup>C</sup> production in differentiated cells is connected with changes in the protein localization and the rate of its posttranslational processing. The physiological interpretation of these alterations remains to be elucidated.

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#### **P149 FRET-based whole genome RNAi screen in search of genes involved in PrP<sup>C</sup> expression**

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Although the prion protein has been studied extensively, its physiological role has not been completely clarified. So far, only a few of the numerous hypothetical functions of PrP<sup>C</sup> could be empirically demonstrated and further investigations are necessary to pin down its enigmatic role. In addition, PrP<sup>C</sup> plays a crucial role in prion disease as a substrate for the formation of PrP<sup>Sc</sup>. The detection of a targetable proteins to downregulate the expression of PrP<sup>C</sup> might pave the way for a possible prolongation of the progression of prion diseases. To approach this subject in a broad and unbiased way, we set up a cell-based RNAi screening platform in which PrP<sup>C</sup> expression levels are displayed. In the human U251-MG glioblastoma cell line, we aim to target the downregulation of the whole genome through siRNAs and 72 hours following transfection, changes in PrP<sup>C</sup> expression by the use of two antibodies targeting different epitopes of PrP<sup>C</sup> coupled to FRET-donor and acceptors, will be assessed. A first test cassette composing of positive controls and negative controls only at a concentration of 5nM yielded a Z-factor of 0.766 demonstrating the feasibility of the approach. Thereby, we are trying to map genes interacting with and involved in the regulation of PrP<sup>C</sup> expression. Such an approach bears the potential to discover novel interaction partners of PrP<sup>C</sup>, which will allow the generation of new hypotheses about the physiological function and might offer possible ways of regulating the expression of the prion protein.

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#### **P150 PrP<sup>C</sup> and EGFR: a multimolecular complex involved during neuronal differentiation of human dental pulp-derived stem cells**

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Aims. The expression of PrP<sup>C</sup> makes mesenchymal stem cells good candidates to develop *in vitro* system for the study of prion infectivity and multiplication. Previous works suggest that lipid rafts and their components, as gangliosides, are essential for neuronal differentiation of different type of stem cells. Since PrP<sup>C</sup> is constitutively expressed in lipid rafts and in a wide variety of stem

cells, the purpose of this study is to investigate the possible role of PrP<sup>C</sup> during neuronal differentiation of human dental pulp-derived stem cells (hDPSCs).

**Methods.** hDPSCs were isolated from third molar of healthy adult subjects (13 to 19 years old) and they were maintained in Dulbecco's Modified Eagle's Medium low glucose, containing 100 units/ml penicillin, 10 mg/ml streptomycin, plus 0,1% amphotericin plus foetal bovin serum (FBS) qualified 10%, at 37°C in humidified CO<sub>2</sub> atmosphere. All samples were collected with informed consent of the patients according to ethics considerations and after subscribed suitable set of forms and with the approval of the ethics committee.

**Results.** hDPSCs express multipotent mesenchymal stromal specific surface antigens, such as CD44, CD90, CD105 and STRO1, but they are negative for hematopoietic markers, such as CD14 and CD19. Moreover, after stimulation with EGF/bFGF, hDPSCs reduce their growth and, after two weeks, it is possible to observe neurites outgrowth and the expression of specific neuronal markers, such as B3-tubulin and NFH. Also, we showed that hDPSCs express precociously PrP<sup>C</sup> at low concentration and its expression increases after two weeks of treatment with EGF/bFGF. Then, we analyzed the association of PrP<sup>C</sup> with gangliosides and EGF receptor (EGF-R) during neuronal differentiation process. In untreated hDPSCs, PrP<sup>C</sup> associate constitutively with GM2 while for GD3 only after neuronal differentiation. Otherwise, EGF-R associate weakly in untreated hDPSCs while it is more markedly expressed after neuronal differentiation. To analyze the functional role of PrP<sup>C</sup> in the signal pathway mediated by EGF/EGF-R, a siRNA PrP was applied to ablate PrP<sup>C</sup> and its function. The treatment with siRNA PrP significantly prevented Akt and ERK1/2 phosphorylation induced by EGF. Moreover, siRNA PrP treatment significantly prevented neuronal-specific antigens expression induced by EGF/bFGF, indicating that cellular prion protein is essential for EGF/bFGF-induced hDPSCs differentiation.

**Conclusions.** In this study we have analyzed the role of PrP<sup>C</sup> in hDPSCs isolated from third molars of health patient. Taken together, our results suggest that PrP<sup>C</sup> interacts with EGF-R within lipid rafts, playing a role in the multimolecular signaling complexes involved in hDPSCs neuronal differentiation.

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## **P151 Silencing prion protein inhibits the proliferation of U87 glioblastoma cell line**

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Cellular prion protein (PrP<sup>C</sup>) may exert neuro- and cyto-protective functions. PrP<sup>C</sup> overexpression protects cultured neurons and also tumor cell lines from damages caused by various pro-apoptotic stimuli including chemotherapy agents. The aim of this work is to investigate the role of PrP<sup>C</sup> protein in the proliferation of U87 GBM cells and its connection with the resistance to chemotherapy agents. PRNP gene in U87 cell line was silenced to determine its effects on cell proliferation and chemoresistance. A decrease of 80% in protein level was obtained after 48h of treatment with the transfection reagent. The effect of PRNP knockdown lasts and the PrP<sup>C</sup> protein expression level doesn't recover even after 5 days of the withdrawal of the transfection. Silencing of the PRNP gene in U87 cells reduces cell proliferation in a time dependent manner according to MTT and Alamar blue proliferation assays. In addition, the number of positive cells with proliferation marker ki67 were significantly reduced in knockdown cells when compared with scramble cells after 5 days. Furthermore, the molecular effects on apoptosis induced by PrP<sup>C</sup> silencing were investigated using U87 cells by analyzing apoptotic marker Annexin V. The results indicated that apoptosis was not induced after PRNP gene downregulation. Regarding the resistance of U87 cells to treatment by chemotherapeutic agents, we observed that the treatment

by TMZ and BCNU do not have much effect on cell viability in a dose dependent manner in the knockdown cells. These findings suggest that the elimination of PrP<sup>c</sup> in U87 can inhibit the proliferation of cells, but is not related to the resistance of cells caused by chemotherapeutic agents.

Key words: Glioblastoma, U87, Prion protein, Proliferation, Apoptosis.

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### **P152 Cell biological, structural and mechanistic aspects of the ADAM10-mediated shedding of the prion protein**

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Proteolytic processing of the prion protein (PrP) is evolutionary conserved, indicating biological relevance of these cleavages and intrinsic functions of the resulting fragments. In fact, increasing evidence suggests that the latter fragments are involved in processes like neuroprotection, myelin maintenance or axon guidance, and may even act as regulators of immune responses and tumorigenesis. Moreover, the very C-terminal ADAM10-mediated release of almost full-length PrP impacts on the course of prion disease in mice. Here, we provide our most recent data on mechanistic and structural aspects influencing this shedding and consider its therapeutic potential. We recently generated an antibody for the sensitive and specific detection of shed PrP. Using this antibody, we analysed shedding efficiency of several PrP mutants with altered N-glycosylation, anchorage and/or localization. The flexible N-terminus, the glycosylation state and the type of membrane anchorage of PrP turned out to be important structural elements influencing the shedding. We also demonstrate manipulation of PrP shedding by administration of ADAM10 inhibitors and stimulators. Of note, inhibition of ADAM10 led to an increased release of PrP via exosomes, whereas impairment of lysosomal transport and degradation resulted in increased ADAM10-mediated shedding. This indicates the importance of tightly controlled PrP levels at the cell surface and suggests shedding as a relevant mechanism in a compensatory network ensuring PrP membrane homeostasis. Finally, as direct pharmacological targeting of ADAM10 might cause severe side-effects due to the multitude of ADAM10 substrates throughout the body, we also addressed effects of a more PrP-directed approach to manipulate the shedding and present an update in this regard.

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### **P153 The cellular prion protein is embedded in a molecular environment tasked with modulating crosstalk between transforming growth factor $\beta$ and integrin signaling**

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At times, it can be difficult to discern if a lack of overlap in reported interactions for a protein-of-interest reflects differences in methodology or biology.

A case in point is the prion protein (PrP), best known for its central role in prion disorders, for which more than two dozen interactors were reported, yet there is little consensus regarding their importance for understanding the biology of PrP. In such instances, systematic analyses of protein-protein networks across diverse paradigms can provide valuable insights.

Here, we interrogated the PrP interactome in four distinct mouse cell lines. Analyses made use of identical affinity capture reagents and sample processing workflows. To enable the discrimination of specific from non-specific binders, negative controls were generated from PrP knockout lines of the respective cell models, and the relative levels of peptides were quantified with the help of isobaric labels.

The study uncovered 26 proteins, which reside in proximity to PrP. All of these proteins are predicted to have access to the outer face of the plasma membrane, and approximately half of them were not reported to interact with PrP before. Strikingly, although several proteins exhibited profound co-enrichment with PrP in a given model, except for Ncam1, no protein was highly enriched in all four PrP-specific interactome datasets. However, Gene Ontology analyses revealed a shared association of the majority of PrP candidate interactors with a cellular biology at the intersection of Tgf- $\beta$  and integrin signaling.

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#### **P154 A conditional PrP<sup>C</sup> allele reveals a gateway to alternative folding - role of vitamin B12**

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**Introduction:** The natively unstructured N-terminal region of wild type (WT) PrP acquires interchangeable structures upon copper ion interactions with 4 binding sites in the octapeptide repeat (OR) region; these structures are called Components 1, 2 and 3 (Chattopadhyay *et al*, J. Am. Chem Soc, 2005). We hypothesized that different N-terminal structures of the OR region modify the conformation of the full-length molecule and hence relate to physiological functions of PrP<sup>C</sup>. Accordingly, we constructed transgenic (Tg) mice expressing PrP allelic forms called S1 and S3 that are predisposed to component 1 and 3 conformations, respectively (Lau *et al*, EMBO Mol Med, 2015). In further studies described here, stably transfected RK13 cells expressing S3-PrP, S1-PrP or WT-PrP transgenes were cultured in different media and then profiled for biochemical and cell biological properties of the corresponding PrP<sup>C</sup> forms.

**Results:** Expressed in RK13 cells grown in OptiMEM™ medium, S3-PrP becomes spontaneously proteinase K resistant (50  $\mu$ g/ml PK); this effect was absent for WT and S1 PrP grown under the same conditions, or for S3-PrP cells grown in DMEM medium. PNGase F digestion produced revealed two proteinase K-resistant C-terminal fragments for S3-PrP, of similar gel mobility to protease-sensitive C1 and C2 fragments produced by physiological endoproteolysis of PrP<sup>C</sup>.

Deconstruction of OptiMEM™'s composition by induction coupled mass spectrometry and mixing experiments defined vitamin B12 (cobalamin), a cobalt-containing nutritional co-factor, as necessary and sufficient for protease-resistance. Exposure of S3-PrP to cobalamin was accompanied by formation of PrP complexes measured in a filter-trap assay and cellular puncta identified by PrP immunostaining. Changes induced in S3-PrP could be reversed by switching to DMEM medium or by use of Ca-EDTA chelator, while addition of Cu(II) ions synergized with cobalamin effects. Lastly, Tg.S3-PrP mice accumulated higher levels of brain cobalt than control Tg lines.

**Conclusion:** We conclude that when the OR region adopts a component 3 conformation, it opens a gateway to restructuring of the whole PrP<sup>C</sup> molecule. Beyond the OR region, occupancy of these alternatively folded forms enabled by cobalamin binding involves *cis* interactions between a downstream site and the globular domain. Protease resistant fragments formed by S3-PrP bear some similarity to two fragments observed in the brain tissue of a subset of sporadic Creutzfeldt-Jakob disease patients and to PK-resistant C1 PrP described in cattle. While formal relationships between component 3 PrP and prion disease pathogenesis remains to be established, our data imply cobalamin interactions feature in the physiology of cellular PrP.

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### **P155 Identification of cellular factors required for prion susceptibility**

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The overarching goal of this work is to identify and understand the role of cellular factors in prion susceptibility. Our central hypothesis, that unidentified factors regulate disease susceptibility and pathogenesis, is based on widespread observations that PrP<sup>C</sup>, while necessary, is not sufficient for prion transmission and neurodegeneration. We demonstrate that clonal variants of RK13 rabbit kidney epithelial cells engineered to express PrP from different species (either deer, elk, or sheep) are variably susceptible to prion infection. Starting from cloned, persistently infected cells, we isolated clonal derivatives that lost prion infection. While the majority of such “cured” (C) clones are subsequently re-infectable (susceptible, or S), a subpopulation is completely resistant (R) to prion reinfection, despite expressing PrP. Since they derive from an infected parental clone, we surmise that this phenotype reflects a genetic change in R from S variants. Using primary mouse embryo fibroblast (MEF) cells that are susceptible to infection with mouse-adapted 22L prions, and we isolated and characterized C, S and R MEF clones using a similar strategy. Using RNA sequencing (RNAseq), we compared the transcriptomes of three sets of RK13- and two sets of MEF-based S and R clones. We identified two differentially expressed genes that were common to all five independent analyses. Specifically, expression of selenophosphate synthetase 1 (Sps1) is uniformly decreased by ~ 2-fold in S compared to R cells, while expression of neurobeachin-like 1 protein gene (NBEAL1), is consistently increased ~ 1.5-fold in S compared to R cells. Transcript changes were verified using real-time PCR, and were reflected in corresponding alterations in protein expression. Sps1 is involved in recycling L-selenocysteine, which is required for further synthesis of selenoproteins, many of which are important in regulation of redox homeostasis. NBEAL1, which is highly expressed in brain, is member of a family of a large family of eukaryotic proteins containing a highly conserved Beige and Chediak-Higashi (BEACH) domain, which is crucial for vesicle trafficking, membrane dynamics and receptor signaling. We are currently exploring the hypothesis that Sps1 and NBEAL1 are candidate susceptibility factors for prion infection using CRISPR/Cas9 gene editing techniques and transgene overexpression approaches.

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## V. Brain junk-clearing mechanisms

### **P156 Phosphorothioate oligonucleotides: From a possible prion therapy to a novel loss of function tool**

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Phosphorothioate oligonucleotides demonstrated a low  $IC_{50}$  for PrP<sup>Sc</sup> in mammalian cell lines and indicated a possible therapy in preclinical trials. Sets of experiments led us to generate a novel loss of function technology (LOFT) in mammalian cell lines that is affordable, rapid, easy to use, and specifically down-regulates membrane proteins (MP) and their variants (MPV), without directly affecting its transcript, in a reversible manner. These attributes enable unique kinetic assay performances in a physiologically relevant manner, providing broad applicability including target validation, drug discovery, drug resistance analysis, biomarker identification, and functional analysis. MPV's are associated with disease progression. However, studying MPV's in their physiological environment and, specifically, targeting the expression of a particular MPV isoform is crucial, yet difficult to achieve. The LOFT technology helps overcome these obstacles. This technology was developed for cancer-related MPV, and we are aiming to test the adaptation of this technology to MPV related to neurodegenerative disorders including the effect of various PrP isoforms on prion infectivity.

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### **P157 Spermine increases acetylation of tubulins and facilitates autophagic degradation of prion aggregates**

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Autolysosomal dysfunction and unstable microtubules are hallmarks of chronic neurodegenerative diseases associated with misfolded proteins. Investigation of impaired protein quality control and clearance systems could therefore provide an important avenue for intervention. To investigate this we have used a highly controlled model for protein aggregation, an in vitro prion system. Here we report that prion aggregates traffic via autolysosomes in the cytoplasm. Treatment with the natural polyamine spermine clears aggregates by enhancing autolysosomal flux. We demonstrated this by blocking the formation of mature autophagosomes resulting in accumulation of prion aggregates in the cytoplasm. Further we investigated the mechanism of spermine's mode of action and we demonstrate that spermine increases the acetylation of microtubules, which is known to facilitate retrograde transport of autophagosomes

from the cellular periphery to lysosomes located near the nucleus. We further report that spermine facilitates selective autophagic degradation of prion aggregates by binding to microtubule protein Tubb6. This is the first report in which spermine and the pathways regulated by it are applied as a novel approach towards clearance of misfolded protein and we hypothesise that this will have important implication for the broader family of protein misfolding diseases.

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#### **P158 Do changes in Hsp110 activity modify prion infection?**

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Mammalian heat shock protein 110 (Hsp110) family members cooperate with Hsp70 and Hsp40 to disaggregate misfolded proteins. Efficient *in vitro* disaggregation of  $\alpha$ -synuclein and artificially denatured proteins by this tri-chaperone system have recently been reported. In yeast, it is well established that the disaggregase formed by Hsp70 and Hsp104 (which has no mammalian homolog) affects the maintenance of prion phenotypes. We hypothesise that the mammalian tri-chaperone system fulfils a similar function by participating in prion fragmentation and disaggregation. Our aim is to test this by altering Hsp110 activity in prion-infected cultured cells and in prion-infected mice. Here, we show that transient knockdown and overexpression of Hsp110 family members in ScN2a cells alter PrP<sup>Sc</sup> levels in a manner reminiscent of Hsp104 modulation in yeast. We also describe the generation of a new Hsp110 knockout N2a cell line as well as other ongoing work including the generation of cell lines stably overexpressing Hsp110 and a prion bioassay in Hsp110 overexpressing mice.

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#### **P159 The neurotoxicity *in vitro* of prion protein fragment 90-231 is mediated by the impairment of autophagolysosomes resolution and can be reverted by autophagy enhancers**

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Protein misfolding and impairment of proteostasis appear as unifying pathogenic traits of prion diseases, Alzheimer diseases, alpha-synucleinopathies and other neurodegenerative conditions in which generation of soluble oligomers of amyloidogenic peptides correlates with neuronal death and glial activation. Consequently, the investigation of neuronal proteostatic mechanisms has become crucial to hypothesize neuroprotective therapies against cerebral amyloidosis. In particular, autophagy-dependent protein degradation is now emerging as major mechanism of cell



survival in response to accumulation of misfolded proteins. In this work we have induced cell death of mesencephalic cell line A1 by prion protein-derived peptide PrP90-231 whose property to form insoluble and protease-insensitive clusters into acidic vesicles was previously described. We observed that PrP90-231 induced A1 cell death and accumulates into acidic vesicles that revealed, at electron microscopy analysis, hallmarks of autophagosomes and autophagolysosomes. The analysis of cytoplasmic expression of autophagy-related proteins LC3I-II, beclin 1 and P62, conformed morphological observations and indicated that in the presence of PrP90-231 autophagolysosomes accumulation occurred, likely because of the protease-insensitivity of the peptide. Under these circumstances, the expression of lysosomal cathepsin D increased, along with its cytosolic diffusion and mitochondrial depolarization. Remarkably, pharmacological induction of autophagy by pharmacological blockade of mTOR kinase or trophic factor deprivation restored resolution of autophagolysosomes, inhibited intracellular PrP90-231 accumulation and reduced neuronal death. Altogether, these data indicate that PrP90-231 produces cytosolic diffusion of hydrolytic enzymes and cell death through the impairment of autophagy progression, being such process crucial to allow A1 survival. Importantly, restoring autophagolysosomes resolution by pharmacological enhancement of autophagy could represent a promising neuroprotective strategy in the therapy of neurodegenerative proteinopathies.

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#### **P160 Oxidative stress and mitochondrial dysfunction in genetic prion disease: a target for preventive treatment?**

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Oxidative stress induced mitochondrial malfunction is a common feature in the advance stages of neurodegenerative conditions associated with misfolded protein accumulation, as is the case for prion diseases. In this work, we examined if this is also the case in the early/subclinical stages of disease. To this effect, we tested the activity and expression of mitochondrial markers in the subclinical and clinical state of TgMHu2ME199K mice, modeling for genetic prion disease. These mice are born healthy, presenting initial clinical disease at 4-6 months of age, then progressive deterioration to a terminal stage at 12-14 months. We found that mitochondrial activity and ROS production were increased at 1.5 months of age while most of the COX (cytochrome oxidase) IV1 isoform was replaced by COXIV2, an isoform which can function under high levels of ROS as well as toxic and degenerative conditions. At advanced disease, brain mitochondrial function declined significantly, concomitant with increased accumulation of disease related PrP and decreased levels of the 20S proteasome. Administration to TgMHu2ME199K mice of Nano-PSO, a brain targeted antioxidant which delays disease advance without reducing PrP levels, significantly corrected functional and biochemical mitochondrial abnormalities at both time points. These results suggest that mitochondrial malfunction may precede clinical disease by months, suggesting neuronal death occurs only when the correction mechanisms are no longer functional. This also indicates that brain targeted reduction of ROS levels may be an appropriate strategy for preventive treatment.

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## VI. PrP<sup>Sc</sup> prions: human disease

### P162 Remote Assessment of Human Prion Diseases for Research and Surveillance

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#### Introduction

The rarity, rapidity, neurological debility, and geographic dispersion of human prion disease present challenges for clinical research studies and surveillance activities. Teleneurology, which uses videoconference technology to assess patients, is widely used in other neurological diseases such as stroke and Parkinson's disease. We present results from a feasibility study for conducting teleneurology evaluations of prion disease subjects for the purposes of conducting research and surveillance.

#### Methods

Thirty-seven subjects from 27 states in the U.S.A. were given a choice of in person evaluation, teleneurology evaluation, or medical record review only in order to determine subject preference. Subjects were able to participate in the study using any internet connected device with a camera and microphone using secure videoconference software (Cisco Jabber). Standardized medical, surgical, family, and acquired prion disease risk factor histories were collected. Standardized assessment instruments were used to assess cognition (Telephone Interview for Cognition Scale), functional ability (MRC Prion Scale), and neuropsychiatric symptoms (Neuropsychiatric Inventory-Questionnaire). A standardized neurological examination adapted from the PRION-1 trial was also used.

#### Results

Subjects overwhelmingly chose to participate in the teleneurology arm of the study (95%). The majority of subjects participated in the study while at home (84%). Twenty-seven percent of subjects underwent more than one teleneurology evaluation. Subjects found it satisfying, easy to use, and would recommend it to future participants. Video quality and subject cooperation varied. Subjects at various degrees of severity could be assessed through this modality. Data regarding cognition, daily functioning, neuropsychiatric symptoms, and neurological status could be reliably collected through teleneurology in this population. A high percentage of subjects underwent post-mortem evaluation (95%) and a fair number of subjects' clinicians reached out to study staff to learn more about prion disease (35%).

#### Discussion

We present data that demonstrate the feasibility of conducting remote teleneurology assessments of prion disease subjects, which was well accepted and able to derive important historical and longitudinal clinical data. Ease of access may affect assessment quality. Preliminary results from our study demonstrate that remote assessment may facilitate clinician engagement and autopsy rates. Teleneurology use in research and surveillance activities of prion disease patients should

be further explored. Future studies will explore the reliability and validity of assessment instruments used in the teleneurology program.

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**P163 Enhanced Creutzfeldt-Jakob Disease Surveillance in the Older Population: an in-depth biochemical and neuropathological analysis of 100 donors to the Edinburgh Brain Bank**

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Creutzfeldt-Jakob disease (CJD) is the prototypic human prion disease that occurs most commonly in sporadic and genetic forms, but it is also transmissible. Variant Creutzfeldt-Jakob disease (vCJD) was first identified in 1996 in the United Kingdom (UK), and results from human exposure to the bovine spongiform encephalopathy agent. There is a concern that UK national surveillance mechanisms might be missing cases of vCJD, or other forms of human prion disease, particularly in the older population, perhaps due to atypical clinical characteristics of disease in these patients. To address this, we conducted in-depth biochemical and neuropathological screening of brain tissue donations to the Edinburgh Brain Bank, for evidence of prion disease. Tissue from 100 donors, aged 65 or over, were examined. To maximise the sensitivity and range of this protocol for detecting prion disease, we employed five biochemical tests, based on different principles for detecting the abnormal, disease-associated prion protein, PrP<sup>Sc</sup>, including *in vitro* conversion assays (RT-QuIC and PMCA) for the amplification of PrP<sup>Sc</sup>. Frozen samples from six defined brain regions from each donated brain were analysed. None of the 100 patients examined showed evidence for prion disease or the presence of PrP<sup>Sc</sup> in the brain regions examined. To date this study has not uncovered a significant under-ascertainment of vCJD, or prion diseases in general, in the older population in Scotland. However, to fully assess the incidence of undiagnosed prion disease in the older population larger studies with high-throughput protocols will be required.

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**P164 Does the RT-QuIC seeding component present in different sCJD samples generate different RT-QuIC end-products?**

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Real Time Quaking Induced Conversion (RT-QuIC) is an *in vitro* prion seeded aggregation assay in which minute amounts of a prion seed promote the misfolding and aggregation of the recombinant prion protein (rPrP), termed RT-QuIC substrate. The first generation diagnostic RT-QuIC assay, which employs full-length recombinant hamster PrP (FLHa-rPrP) as a substrate, is highly sensitive and specific in identifying biological samples of sporadic Creutzfeldt-Jakob (sCJD) disease, the most common human prion disease. The kinetics of aggregation of the RT-QuIC substrate can be monitored by a shift in the fluorescence emission of the amyloid-specific dye Thioflavin-T (ThT), incorporated in the RT-QuIC reaction. The RT-QuIC end-products are rPrP aggregates that bind ThT and are partially resistant to proteases. Published evidence shows

that RT-QuIC end-products, following limited enzymatic proteolysis and separation by SDS-PAGE, show different patterns of rPrP fragments, according to the prion seed used.

Our aim is to determine whether first generation diagnostic RT-QuIC is able to discriminate the different sCJD subtypes as defined by the combination of *PRNP*-codon 129 genotype and protease K resistant PrP (PrP<sup>res</sup>) types. To do so, we employed first generation diagnostic RT-QuIC, seeded with sCJD samples, to produce rPrP aggregates. These aggregates were then subject to limited proteolysis and western blot analysis, to determine an epitope map of surviving protease resistant fragments. Human sCJD brain homogenate and human sCJD CSF samples used in this study were obtained with consent from a cohort of patients encompassing the most common sCJD subtypes. The immunodetection of partially resistant products, generated by RT-QuIC seeded with the various sCJD samples, was assessed using six commercially available monoclonal antibodies, including those routinely used for the molecular typing of sCJD molecular subtypes. This analysis of the RT-QuIC end-products may provide insights into the strain-specific misfolding of rPrP, and it may contribute to the differential diagnosis of human prion disease subtypes.

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### **P165 Evaluation of truncated versus full length hamster recombinant PrP as a substrate for CSF RT-QuIC in the diagnosis of sporadic CJD subtypes**

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#### Aims:

CSF RT-QuIC is a clinical diagnostic test for sporadic CJD (sCJD) which often makes use of full length Hamster (23-231) prion protein (FL Ha PrP) as a substrate. Recent reports suggest that the test can be made faster using a truncated version (90-231) of the prion protein as a substrate (Trunc Ha PrP) without an appreciable decrease in sensitivity.

In this study we compared the sensitivity and lag time (i.e. time to positivity) of the CSF RT-QuIC assay using FL Ha PrP and Trunc Ha PrP.

#### Methods:

The protein substrates were produced in the National CJD Research & Surveillance Unit. Both protein production and RT-QuIC used previously published conditions (McGuire *et al* 2016 and Foutz *et al* 2017).

CSF samples from 57 neuropathologically confirmed sCJD patients with known codon 129 genotype and PrP<sup>Sc</sup> isotypes were analysed by RT-QuIC on a FLUOStar Omega. Of these 57 sCJD patients: 18 (7M:11F; mean age  $\pm$  SD: 72.7  $\pm$  9.4 years) were MM1; 9 (7M:2F; mean age  $\pm$  SD: 69.0  $\pm$  8.6 years) were MM2; 6 (2M:4F; mean age  $\pm$  SD: 66.2  $\pm$  7.6 years) were MV1; 7 (5M:2F; mean age  $\pm$  SD: 68.3  $\pm$  7.3 years) were MV2; 2 (2M:0F; mean age  $\pm$  SD: 50.5  $\pm$  34.7 years) were VV1 and 15 (4M:11F; mean age  $\pm$  SD: 67.3  $\pm$  9.6 years) were VV2.

For each sCJD isotype, the overall sensitivity and lag time were noted.

#### Results:

Sensitivities: MM1 (100% FL Ha PrP: 84% Trunc Ha PrP), MM2 (82% FL Ha PrP : 55% Trunc Ha PrP), MV1 (83% FL Ha PrP : 50% Trunc Ha PrP), MV2 (86% FL Ha PrP: 86% Trunc Ha PrP), VV1 (50% FL Ha PrP : 0% Trunc Ha PrP), VV2 (100% FL Ha PrP : 69% Trunc Ha PrP).

Lag times (hours  $\pm$  SD): MM1 (FL Ha PrP  $22.4 \pm 8.9$  hrs : Trunc Ha PrP  $7.3 \pm 2.6$  hrs), MM2 (FL Ha PrP  $20.4 \pm 13.6$  hrs : Trunc Ha PrP  $6.8 \pm 3.1$  hrs), MV1 (FL Ha PrP  $27.3 \pm 8.9$  hrs : Trunc Ha PrP  $11.8 \pm 9.4$  hrs), MV2 (FL Ha PrP  $21.4 \pm 7.7$  hrs : Trunc Ha PrP  $5.5 \pm 3.1$  hrs), VV1 (FL Ha PrP  $14.0 \pm 2.0$  hrs : Trunc Ha PrP  $20.0 \pm 8.0$  hrs), VV2 (FL Ha PrP  $14.8 \pm 6.8$  hrs : Trunc Ha PrP  $6.4 \pm 1.1$  hrs)

#### Discussion:

RT-QuIC using FL Ha PrP has previously been shown to be a sensitive and specific test for sporadic CJD. The data shown here suggest that Trunc Ha PrP gives a faster test but had a lower sensitivity.

Interestingly, the sensitivities for both the FL Ha PrP and Trunc Ha PrP substrates measured in these sample populations are lower than those reported elsewhere. Causes for the low sensitivity may be an artefact of small sample population, the age of samples (i.e. CSFs undergoing an extended number of freeze-thaw cycles) or batch-to-batch variation in the quality of protein substrates used. Work is ongoing to increase the power of this study by increasing the sample number and quantifying the 'batch-to-batch' variation in protein quality.

Despite these factors, the Trunc Ha PrP gave a lower sCJD sensitivity than FL Ha PrP in 5 out of the 6 isotypes. This work suggests that the Trunc Ha PrP substrate may have an overall lower sensitivity than the FL Ha PrP substrate when used in CSF RT-QuIC.

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#### **P166 Characterization of CJD strain profiles in venison consumers and non-consumers from Alberta and Saskatchewan**

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Chronic wasting disease (CWD) is spreading rapidly through wild cervid populations in the Canadian provinces of Alberta and Saskatchewan. While this has implications for tourism and hunting, there is also concern over possible zoonotic transmission to humans who eat venison from infected deer. Whilst there is no evidence of any human cases of CWD to date, the Canadian CJD Surveillance System (CJDSS) in Canada is staying vigilant. When variant CJD occurred following exposure to BSE, the unique biochemical fingerprint of the pathologic PrP enabled a causal link to be confirmed. However, we cannot be sure what phenotype human CWD prions would present with, or indeed, whether this would be distinct from that seen in sporadic CJD. Therefore we are undertaking a systematic analysis of the molecular diversity of CJD cases of individuals who resided in Alberta and Saskatchewan at their time of death comparing venison consumers and non-consumers, using a variety of clinical, imaging, pathological and biochemical markers. Our initial objective is to develop novel biochemical methodologies that will extend the baseline glycoform and genetic polymorphism typing that is already completed by the CJDSS. Firstly, we are reviewing MRI, EEG and pathology information from over 40 cases of CJD to select clinically affected areas for further investigation. Biochemical analysis will include assessment of the levels of protease sensitive and resistant prion protein, glycoform typing using 2D gel electrophoresis, testing seeding capabilities and kinetics of aggregation by quaking-induced conversion, and determining prion oligomer size distributions with asymmetric flow field

fractionation with in-line light scattering. Progress and preliminary data will be presented. Ultimately, we intend to further define the relationship between PrP structure and disease phenotype and establish a baseline for the identification of future atypical CJD cases that may arise as a result of exposure to CWD.

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**P167 Evaluating sensitivity and specificity of multiple neurodegenerative proteins in the diagnosis of CJD using the deep learning tool**

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Creutzfeldt-Jakob disease (CJD) is diagnosed based on clinical criteria, magnetic resonance imaging, electroencephalography, and increased levels of 14-3-3 protein in cerebrospinal fluid (CSF). Sporadic CJD (sCJD) is the most common human prion disease. A CJD diagnosis can only be confirmed by abnormal protease-resistant prion protein accumulation in postmortem brain tissue. Many biomarkers, including 14-3-3, neuron-specific enolase, and tau, have been investigated for use in the differential diagnosis of sCJD. The relationships between the disease and CSF proteins such as 14-3-3, tau,  $\alpha$ -synuclein (a-syn), and  $\beta$ -amyloid (1-42) have been studied for the use of these proteins as early premortem diagnostic biomarkers. However, no single CSF biomarker or biomarker combination has shown significantly high sensitivity or specificity in Korean patients with CJD. Recently, deep learning (DL), which is a family of machine learning methods and agnostic tools, have gained attention in neurodegenerative disease research. In this study, we evaluated their value using multiple CSF biomarkers and DL to improve their sensitivity and specificity in Korean patients with sCJD. Enzyme-linked immunosorbent assays were performed on phospho-tau (p-tau), total-tau (t-tau), a-syn,  $\beta$ -amyloid (1-42), and 14-3-3 western blots from 56 patients with CJD and 263 non-CJD patients in Korea. In the DL analysis, 269 of 319 CSF samples were used for training and test sets, and the remaining 50 were used as a validation set. We designed a modular ensemble of 10, 20, 30, and two deep neural networks to distinguish patients with and without CJD. We ran 400 epochs of the Nadam optimizer with binary cross-entropy loss. The best performing deep neural network model demonstrated 90% accuracy, 83.3% sensitivity, and 92.1% specificity for three proteins combination (t-tau, p-tau, and a-syn). However, without DL, the best CSF biomarker was t-tau with 75% sensitivity and 84.2% specificity. Premortem diagnoses of CJD performed using combinations of multiple CSF markers with DL showed better accuracy than using a single CSF biomarker without DL in Korean patients with CJD. Additional studies are required to improve the performance of premortem diagnoses with larger sample sizes from various countries around the world.

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**P169 Strain specific PrP seeding activity and biomarker profile in MM1/VV2 subtype specific humanized-CJD Mice**

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To apprehend the early diagnosis, altered disease progression rates and molecular signature of human prion strains, we analysed time course depended seeding/amplification activity of prions in mice by real-time quaking-induced conversion (RT-QuIC) assay. In this study, tg340, tg361 (expressing about four fold of human PrP-M129 and PrP-V129, respectively) and in tg650 (expressing about six fold human PrP-M129) mic were innoculated with infectious prion strains from human CJD- MM1 and VV2 brains tissues. Altogether, we analysed 188 brain (cortex and cerebellum regions) samples from confirmed CJD infected mice or control mice at pre-clinical and clinical stages of the disease. Interestingly, we found brain region specific and PrP strain specific differential seeding activity at pre-clinical stage of the disease in CJD- MM1 and VV2 infected mice. The lag phase between the positive response showed up from 7.5 and 24.5 hours in all regions and stages of the disease. However, in cortex region, the CJD- MM1 infected tg340 mice showed longer lag phase approximately 24.5 hours and CJD-VV2 infected tg341 mice showed minimal seeding response and rfu signal rate. On the other hand, in cerebellum, VV2 clinical stage mice showed smaller lag phase and VV2 early preclinical stage showed significant minimal rfu signal rates. Relative expression of functionally categorized 1100 proteins demonstrate strain specific preclinical signature of proteins. In conclusion, our findings demonstrated for the first time a key pre-clinical response of strain specific and brain region associated pathway in prion disease.

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## **P170 Morphological glial alterations in different Creutzfeldt-Jakob's disease types**

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According with the neuroinflammation hypothesis gliosis is pointed to play a main role in the neurodegenerative process of prion diseases. Joint to spongiosis and protein deposits, they constitute their histopathological hallmarks. In fact, many efforts have been made in order to determine profiles based on vacuolation and PrPsc accumulation providing identification and characterization of different types of this group of diseases. Since host processing also seems to be involved in these phenotypical profiles, to elucidate glial cells behavior and their morphological alterations might answer some unknown questions about neurodegeneration.

The overall aim of this study is to comparatively analyze the morphological changes of glial cells as involved cell populations in the primary immunological response of the brain from different Creutzfeldt-Jakob disease (CJD) types in two specific brain areas.

Similar immunohistochemical profiles in both encephalic areas were shared regardless the type examined. The results presented in this study support the involvement of glial cells in CJD demonstrated by specific morphological changes that may suggest a direct involvement in prion disease pathogenesis independent of brain area and moreover, CJD type.

To our knowledge, few works focused on the relationship of this cell population and the different CJD types have been developed as an approach to pathogenesis of prion diseases. The results provided here support that all types may share a molecular basis and mechanisms of propagation related with neuroglia.

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### **P171 Prion disease incidence, United States, 2003-2015**

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Introduction. The incidence of invariably fatal prion diseases such as Creutzfeldt-Jakob disease (CJD) in the United States can be estimated by analyzing data from multiple sources.

Methods. Prion disease decedents were identified from restricted-use U.S. national multiple cause-of-death data, via a data use agreement with the National Center for Health Statistics, and from the National Prion Disease Pathology Surveillance Center (NPDPS) database for 2003-2015. Likely decedent matches between the two databases were determined based on a comparison of demographic variables including sex, date of birth, date of death, and state of residence. NPDPS decedents with neuropathological or genetic test results positive for prion disease for whom no match was found in the multiple cause-of-death data were added as cases for incidence calculations; those with negative neuropathology results but with cause-of-death data indicating prion disease were removed. Average annual age-adjusted incidence rates were then calculated using the year 2000 as the standard population.

Results. A total of 5212 decedents were identified as having prion disease during 2003-2015 for an average annual age-adjusted incidence of 1.2 per million population. The annual age-adjusted incidence ranged from 1.0 per million in 2004 and 2006 to 1.4 per million in 2013. The difference in age-adjusted incidence between males and females (1.3 and 1.1 per million, respectively) was significant ( $p < 0.0001$ ). The highest age-adjusted incidence was found among residents of the Midwest (1.3 per million); significantly lower rates were seen in the South (1.1 per million,  $p < 0.0001$ ) and West (1.2 per million,  $p = 0.002$ ). Ten cases during the time period were  $< 30$  years of age for an incidence of 6.2 per billion; only 2 of these very young cases were sporadic forms of prion disease. The incidence among those  $< 55$  and  $\geq 55$  during the time period was 0.2 and 4.7 per million, respectively; incidence among those  $\geq 65$  was 5.9 per million.

Conclusion. Prion disease incidence can be estimated by augmenting mortality data with the results of neuropathological and genetic testing. Higher incidence among older age groups was expected given the median death age of 68 years for CJD cases. Conversely, cases  $< 30$  years of age were extremely rare, and most could be attributed to exogenous factors or the presence of a genetic mutation.



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## **P172 Peripheral Neuropathy in Patients with Prion Disease**

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Prion disease is a fatal progressive neurodegenerative disease due to deposition of an abnormal protease-resistant isoform of prion protein. Typical symptoms include rapidly progressive dementia, myoclonus, visual disturbance and hallucinations. Interestingly, in patients with prion disease, the abnormal protein can also be found in the peripheral nervous system. Case reports of prion deposition in peripheral nerves have been reported. Peripheral nerve involvement is thought to be uncommon; however, little is known about the exact prevalence and features of peripheral neuropathy in patients with prion disease.

We reviewed autopsy-proven prion cases from the National Prion Disease Pathology Surveillance Center that were diagnosed between September 2016 to March 2017. We collected information regarding prion protein diagnosis, demographics, comorbidities, clinical symptoms, physical exam, neuropathology, molecular subtype, genetics lab, brain MRI, image and EMG reports.

Our study included 104 patients. Thirteen (12.5%) patients had either subjective symptoms or objective signs of peripheral neuropathy. Among these 13 patients, 3 had other known potential etiologies of peripheral neuropathy such as vitamin B12 deficiency or prior chemotherapy. Among 10 patients that had no other clear etiology, 3 (30%) had familial CJD. The most common sCJD subtype was MV1-2 (30%), followed by MM1-2 (20%). The Majority of cases were male (60%). Half of them had exposure to wild game. The most common subjective symptoms were tingling and/or numbness of distal extremities. The most common objective finding was diminished vibratory sensation in the feet. Half of them had an EMG with the findings ranging from fasciculations to axonal polyneuropathy or demyelinating polyneuropathy.

Our study provides an overview of the pattern of peripheral neuropathy in patients with prion disease. Among patients with peripheral neuropathy symptoms or signs, majority has polyneuropathy. It is important to document the baseline frequency of peripheral neuropathy in prion diseases as these symptoms may become important when conducting surveillance for potential novel zoonotic prion diseases.

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## **P173 RT-QuIC assay on olfactory brushings in asymptomatic carriers of E200K *PRNP* mutation. An explorative study for establishing when a preventive therapy should be started.**

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Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA.

The main objective of this study is to determine when an eventual preventive therapy should be started in healthy carriers of the glu-to-lys mutation at codon 200 (E200K) of the prion protein gene (*PRNP*). To achieve this goal, we investigated the seeding activity of the prion protein (PrP) by the Real Time QuakingInduced Conversion (RT-QuIC) assay in olfactory mucosa (OM) samples, taken by the non-invasive nasal brushing procedure, from healthy subjects belonging to families affected by genetic CJD. We previously showed that the RT-QuIC test in OM is 100% sensitive and specific in both sporadic and E200K genetic CJD patients (Orrù et al. NEJM 2014). We also found that OM samples resulted RT-QuIC positive in the early phase of disease. We are therefore confident that this test will provide reliable information on the detection of PrP<sup>Sc</sup> in the CNS during a preclinical stage.

71 family members belonging to the cluster area of Calabria region (Italy) agreed to donate blood (for genetic testing) and OM samples (for RT-QuIC assay) for research investigations. Twenty-one healthy members carried E200K mutation. We tested OM samples by RT-QuIC using different recPrP substrates including Ha (23-231 and 90-231), BV (23-231,109M) in different experimental conditions. In OM positive controls from clinically affected E200K patients, we were able to obtain a RT-QuIC positive reaction in few hours with a 100% sensitivity. However, none of the OM sample obtained from E200K mutation carriers resulted positive. A follow-up study is still ongoing.

Acknowledgments: this study has been supported by CJD Foundation.

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#### **P174 Identification and validation of blood-borne protein biomarkers for prion infection**

C. Farren, D. Kurian, A.C. Gill and E. F. Houston

University of Edinburgh.

##### Aims:

Following exposure of the UK population to bovine spongiform encephalopathy (BSE), it has been estimated from the detection of abnormal PrP in appendix tissue samples that up to 1 in 2000 people may be subclinical carriers of variant Creutzfeld-Jakob disease (vCJD). This is a cause for concern due to the potential for iatrogenic transmission via blood/organ donation or surgical instruments, and it is therefore imperative to develop rapid screening tests that can be applied to easily accessible samples such as blood or urine for detection of these “silent” infections. The aim of this project was to identify proteins other than PrP<sup>Sc</sup> that are differentially regulated in the blood of prion-infected sheep, and evaluate their potential for use as diagnostic biomarkers.

##### Methods:

Samples of plasma and buffy coat collected from 9 sheep prior to infection, and 10 months after oral infection with BSE (at least 8 months before the onset of clinical signs), were pooled to form “infected” and “uninfected” samples for comparison. The pooled samples were processed using ProteoMiner™ columns to deplete highly abundant proteins while simultaneously enriching for low abundant proteins, and isotopic labelling was used to distinguish between the two samples and compare levels of protein expression by mass spectrometry. The extent of differential expression was assessed by filtering the median H/L (heavy/light isotopes) and proteins between >1.5 and <0.666 were retained. Differential expression of selected proteins was validated by Western blotting on the original pooled samples, followed by testing on longitudinal samples sets

collected from individual infected and negative control sheep at intervals over the entire time course of infection.

#### Results:

Mass spectrometry analysis identified 153 and 1147 differentially regulated proteins in the plasma and buffy coat datasets, respectively, of which 44 plasma proteins and 124 buffy coat proteins were significantly up- or downregulated. So far, Western blots to determine the relative expression of 3 selected proteins in infected and uninfected plasma pools have confirmed the mass spectrometry results, and validation of additional proteins is ongoing.

#### Conclusions:

We have identified a number of candidate plasma and buffy coat proteins that are significantly up- or down-regulated during preclinical prion infection, and are evaluating their potential sensitivity and specificity as disease biomarkers.

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### **P175 Characterization of the transmission properties of *in vitro* generated FFI and CJD<sup>178</sup> mouse prions**

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#### Background

We have generated transgenic Tg(FFI) and Tg(CJD) mice expressing the mouse PrP homologues of the human D178N/M129 and D178N/V129 mutations linked to fatal familial insomnia (FFI) and genetic Creutzfeldt-Jakob disease (CJD<sup>178</sup>). These mice develop key clinical and neuropathological features of the corresponding human disorders without developing spontaneous prion infectivity (Bouybayoune et al., 2015; Chiesa et al., 2016).

#### Aim

To test whether the mutant PrPs produced in Tg(FFI) and Tg(CJD) mice can be induced to acquire infectious conformations that propagate in mice producing distinct disease phenotypes.

#### Results

Brain homogenates from Tg(FFI) and Tg(CJD) mice were subjected to serial protein misfolding cyclic amplification (PMCA) with or without a RML prion seed. RML-seeded PMCA yielded forms of mutant PrP that were highly PK-resistant (referred to as FFI<sup>RML</sup> and CJD<sup>RML</sup>), while the unseeded reactions did not. To test whether FFI<sup>RML</sup> and CJD<sup>RML</sup> were able to propagate *in vivo* as *bona fide* prions, the PMCA reactions were intracerebrally inoculated in Tga20 mice. The inoculated mice did not develop clinical signs of scrapie, but at the time of sacrifice (> 400 days post-inoculation: d.p.i.) some of them had highly PK-resistant PrP in their brains, suggesting a subclinical prion infection. Brain homogenates from these mice were re-inoculated in Tga20 mice (second passage). The CJD<sup>RML</sup>-inoculated mice showed clinical signs at 357 ± 10 d.p.i, and died at 387 ± 8 d.p.i. (n = 11; mean ± SEM), whereas mice inoculated with FFI<sup>RML</sup> showed clinical signs at 478 ± 20 d.p.i. and died at 509 ± 23 d.p.i. (n = 9; mean ± SEM). The inoculated mice had predominant di-glycosylated PK-resistant PrP in their brains, like humans carrying the D178N/M129 and D178N/V129 mutations.

#### Conclusions

These data indicate that the *in vitro*-converted mutant PrPs from Tg(FFI) and Tg(CJD) mice are able to propagate *in vivo* causing fatal neurological diseases as authentic prions. Analysis are

underway to determine whether FFI<sup>RML</sup> and CJD<sup>RML</sup> represent different prion strains with FFI- and CJD<sup>178</sup>-specific properties.

#### References

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Chiesa, R., Restelli, E., Comerio, L., Del Gallo, F., and Imeri, L. (2016). Transgenic mice recapitulate the phenotypic heterogeneity of genetic prion diseases without developing prion infectivity: Role of intracellular PrP retention in neurotoxicity. *Prion* 10, 93–102.

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#### **P176 Sporadic and Familial CJD - Data from Tel Aviv Medical Center in Israel 2010-2017**

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Background: In Israel, Familial Creutzfeldt-Jakob disease (fCJD) is the most common subtype of CJD, as mutations in the PRNP gene (E200K) are common in Jewish of Libyan and Tunisian ancestry. The objective of this study was to describe the clinical characteristics of CJD patients (familial and sporadic).

Methods: Between the years 2010-2017, 32 patients who were admitted to the Neurological department and were diagnosed with CJD. Clinical data of all patients were collected and are presented here.

Results: 32 patients (M=17,F=15) were diagnosed with CJD. 19 had the E200K mutation. Overall mean age of onset was 62.4 years (SD=9.04) and the duration of disease (from onset of symptoms to death) was 7.3 months (SD=6.8). Mean Tau in CSF was 1625 pg/ml (SD=710). Clinical presentation included cognitive changes, sleep disturbances, gait disorders and sensory deficits. There were no differences between mutation carriers and non-carriers regarding clinical, laboratory and imaging characteristics.

Conclusions: In this sample no differences were observed between the clinical phenotype of familial and sporadic CJD.

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#### **P177 PrP plaques in methionine homozygous Creutzfeldt-Jakob disease patients as a potential marker of iatrogenic transmission**

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## Background

Sporadic Creutzfeldt-Jakob disease (CJD) is widely believed to originate from de novo spontaneous conversion of normal prion protein (PrP) to its pathogenic form, but concern remains that some reported sporadic CJD cases may actually be caused by disease transmission via iatrogenic processes. For cases with methionine homozygosity (CJD-MM) at codon 129 of the *PRNP* gene, recent research has pointed to plaque-like PrP deposition as a potential marker of iatrogenic transmission for a subset of cases. This phenotype is theorized to originate from specific iatrogenic source CJD types that comprise roughly a quarter of known CJD cases.

## Methods

We reviewed scientific literature for studies which described PrP plaques among CJD patients with known epidemiological links to iatrogenic transmission (receipt of cadaveric human growth hormone or dura mater), as well as in cases of reported sporadic CJD. The presence and description of plaques, along with CJD classification type and other contextual factors, were used to summarize the current evidence regarding plaques as a potential marker of iatrogenic transmission. In addition, 523 cases of reported sporadic CJD cases in the US from January 2013 through September 2017 were assessed for presence of PrP plaques.

## Results

We identified four studies describing 52 total cases of CJD-MM among either dura mater recipients or growth hormone recipients, of which 30 were identified as having PrP plaques. While sporadic cases were not generally described as having plaques, we did identify case reports which described plaques among sporadic MM2 cases as well as case reports of plaques exclusively in white matter among sporadic MM1 cases. Among the 523 reported sporadic CJD cases, 0 of 366 MM1 cases had plaques, 2 of 48 MM2 cases had kuru plaques, and 4 of 109 MM1+2 cases had either kuru plaques or both kuru and florid plaques. Medical chart review of the six reported sporadic CJD cases with plaques did not reveal clinical histories suggestive of potential iatrogenic transmission.

## Conclusions

PrP plaques occur much more frequently for iatrogenic CJD-MM cases compared to sporadic CJD-MM cases. Plaques may indicate iatrogenic transmission for CJD-MM cases without a type 2 Western blot fragment. The study results suggest the absence of significant misclassifications of iatrogenic CJD as sporadic. To our knowledge, this study is the first to describe grey matter kuru plaques in apparently sporadic CJD-MM patients with a type 2 Western blot fragment.

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### **P178 Presence of Lewy dots in the brain of patients with Creutzfeldt-Jakob disease**

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Creutzfeldt-Jakob's disease (CJD) is a prion neurodegenerative disorder that rapidly leads to dementia and death. The fundamental event in the biology of prion diseases is a conformational transition in PrP<sup>C</sup> to the disease-causing isoform PrP<sup>Sc</sup>. Accumulation of PrP<sup>Sc</sup> leads to its polymerization into amyloid fibrils that consolidate into toxic oligomers and plaques. Brain deposits of pathological protein aggregates also occur in some neurodegenerative disorders like Alzheimer's and Parkinson's diseases, in which tau and alpha-synuclein aggregates have been consistently found, respectively.

We analyzed the expression of phosphorylated alpha-synuclein in thirteen cases of sporadic Creutzfeldt-Jakob disease (sCJD) and ten age-matched controls in order to determine the existence and location of pathological protein aggregates. We focused our work on the frontal cortex and cerebellum, since they are specially affected in CJD. Single immunohistochemistry for the previous marker was performed to locate, characterize and understand the pathological protein aggregates.

After carefully analyzing the expression of phosphorylated alpha-synuclein, we found alpha-synuclein immunoreactive granular deposits (Lewy dots) in all sCJD brain specimens examined. These inclusions were more prominent in the dentate nucleus and in the cerebellar granular layer than in the frontal cortex, where profuse cytoplasmic inclusions were observed. Surprisingly, we only found Lewy dots and neurites but no Lewy bodies were observed.

The existence of phosphorylated alpha-synuclein cytoplasmic inclusions and Lewy dots in cases of sCJD disease could suggest a common neurodegenerative pathway between CJD and Parkinson disease, since perturbation of alpha-synuclein homeostasis is a common mechanism involved in these two pathologies.

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#### **P179 Application of PMCA for screening of biological therapeutic products for infectious prions**

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Advances in biotechnology have led to the development of a variety of biological therapies for the treatment of diverse diseases. Biological products include cell therapies, vaccines, antibodies, specific proteins, gene therapies, and other products derived from human or animal tissues. Since these products will be administered to humans, it is crucial to test their safety and for the absence of infectious agents. Specifically, prion contamination may pose a threat when using materials derived from humans or animals (e.g. cattle). Indeed, in the past many cases of iatrogenic Creutzfeldt-Jakob disease were caused by the use of biological materials (e.g. human growth hormone) contaminated with prions. Similarly, there have been cases of prion transmission by transfusion of blood obtained from donors at a preclinical stage of prion disease. For this reason, it is important to screen cells and biological materials for the presence of prions. Here we show the utility of the Protein Misfolding Cyclic Amplification (PMCA) technology as a screening tool for the presence of human (vCJD) and bovine (BSE) prions in human cell therapies. First, we demonstrated that PMCA has the capability to detect a single cell infected with prions. For these experiments we used RK13-M7 cells chronically infected with RML. Serial dilutions of an infected cell culture showed that PMCA was able to amplify prions even from an estimated individual cell. Additionally, we determined that PMCA performance was not compromised by the addition of large quantities of cells into the reaction. Finally, we processed a human-derived stem cell line developed for therapeutic use and found them to be PMCA-negative for vCJD and BSE prions. Our findings demonstrate the utility of PMCA as a quality control procedure in the production of biological therapeutics.

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#### **P180 Clinico-pathological analysis of human prion diseases in a brain bank series**

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#### Background and objective:

The Neurological Tissue Bank (NTB) of the Hospital Clínic-Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain is the reference center in Catalonia for the neuropathological study of prion diseases in the region since 2001. The aim of this study is to analyse the characteristics of the confirmed prion diseases registered at the NTB during the last 15 years.

#### Methods:

We reviewed retrospectively all neuropathologically confirmed cases registered during the period January 2001 to December 2016.

#### Results:

176 cases (54,3% female, mean age: 67,5 years and age range: 25-86 years) of neuropathological confirmed prion diseases have been studied at the NTB. 152 cases corresponded to sporadic Creutzfeldt-Jakob disease (sCJD), 10 to genetic CJD, 10 to Fatal Familial Insomnia, 2 to Gerstmann-Sträussler-Scheinker disease, and 2 cases to variably protease-sensitive prionopathy (VPSPr). Within sCJD subtypes the MM1 subtype was the most frequent, followed by the VV2 histotype.

Clinical and neuropathological diagnoses agreed in 166 cases (94%). The clinical diagnosis was not accurate in 10 patients with definite prion disease: 1 had a clinical diagnosis of Frontotemporal dementia (FTD), 1 Niemann-Pick's disease, 1 Lewy Body's Disease, 2 Alzheimer's disease, 1 Cortico-basal syndrome and 2 undetermined dementia. Among patients with VPSPr, 1 had a clinical diagnosis of Amyotrophic lateral sclerosis (ALS) and the other one with FTD.

Concomitant pathologies are frequent in older age groups, mainly AD neuropathological changes were observed in these subjects.

#### Discussion:

A wide spectrum of human prion diseases have been identified in the NTB being the relative frequencies and main characteristics like other published series. There is a high rate of agreement between clinical and neuropathological diagnoses with prion diseases. These findings show the importance that public health has given to prion diseases during the past 15 years. Continuous surveillance of human prion disease allows identification of new emerging phenotypes. Brain tissue samples from these donors are available to the scientific community. For more information please visit:

[www.clinicbiobanc.org/banc-teixits-neurologics/mostres/en\\_index.html](http://www.clinicbiobanc.org/banc-teixits-neurologics/mostres/en_index.html)

### **P181 Heterogeneous phenotype and peripheral prion distribution in a primate model of vCJD blood transmission**

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**Background:** Prevalence studies, that contribute to estimate the risk of blood transmission associated with variant Creutzfeldt-Jakob disease (vCJD), relies on the ability of the BSE agent to replicate in human lymphoid organs before neuroinvasion. Accordingly, the detection of healthy carriers of vCJD infection in the UK, with an estimated prevalence of 1/2,000 i.e. more than 100-fold higher than the prevalence of clinical vCJD, raised concern about a higher risk than previously thought. In cynomolgus macaques and conventional mice, intravenous inoculation of vCJD-derived blood and brain products efficiently transmits vCJD but also unexpected incomplete phenotypes (a myelopathic syndrome in primates) that escape the current diagnosis criteria of prion diseases. Such a combination of experimental models provides an opportunity to evaluate the relationship between peripheral replication and blood infectivity.

**Study design and methods:** Twenty-seven macaques exposed to vCJD-derived blood or brain products through the intravenous route were included in this study. At the terminal stage of their respective clinical phase (17 and 10 macaques developed vCJD or a myelopathic syndrome respectively), the presence of abnormal prion protein (PrP) was studied in their lymphoid organs using immunohistochemical and biochemical approaches, and in blood using amplification techniques (PMCA on plasma and buffy coat samples). Blood infectivity was assessed through intravenous inoculation into conventional and immunodeficient mice.

**Results:** clinically-affected v-CJD primates exhibited a heterogeneous intra- and inter-individual distribution of detectable peripheral replication (among the 10,065 analyzed follicles, &è of them – distributed within (§ of the considered lymph nodes - were found positive), including individuals without any detectable peripheral prion accumulation. In its grand scheme, the global level of peripheral replication correlated to blood infectivity, and PMCA techniques on plasma and buffy coat were able to detect all blood samples that transmitted vCJD to macaque or mouse recipients. Conversely, in myelopathic primates, peripheral replication in lymphoid organs was rarely detected, and incomplete prion phenotypes were transmitted through intravenous route to mice, even in the absence of a functional immune system.

**Cconclusion:** These observations suggest that prevalence studies might underestimate the real magnitude of vCJD healthy carriage. However, risk of vCJD blood transmission should be limited to the donors with the highest peripheral level of infectivity and reduced by using sensitive blood screening tests based on prion amplification in defined situations in transfusion medicine. Conversely, our results suggest that atypical phenotypes may be transmitted independently of the immune system and would escape detection.

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### **P182 Alien limb in sporadic Creutzfeldt-Jakob Disease**



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**Background:** Alien limb syndrome was first described in 1973 by Brion and Jedynak in three patients with corpus callosum tumours. The syndrome was defined as a combination of involuntary, semi-purposeful, limb movements in the absence of volition and has since been reported in other conditions including stroke and neurodegenerative disorders. Whilst alien limb syndrome is recognised in sporadic Creutzfeldt Jakob disease (CJD), description has been limited to individual case reports or small case series.

**Aims:** 1) To establish the frequency of alien limb symptoms in patients with sporadic CJD assessed by the UK National CJD Research and Surveillance Unit (NCJDRSU), and, 2) Describe the clinical features and investigation results of these cases

**Methods:** We reviewed all sporadic CJD cases with features of alien limb syndrome assessed by the NCJDRSU fulfilling the criteria for probable and definite CJD over a 12-year period (October 2005-October 2017).

**Results:** Sixty-nine cases (30 male, 39 female, median age at onset 67 years, median duration of illness 3 months) presented with features of alien limb syndrome from a total of 1025 (6.7% of all cases): (36 probable/33 definite (post mortem confirmed)). Thirteen of these cases (18.8%) presented with isolated alien limb syndrome at onset. All cases developed rapidly progressive cognitive decline. Codon 129 status was analysed in 63% of cases (MM 88.6%, MV 4.6%, VV 6.8%). CSF 14-3-3 was positive in 41/53(77.4%) and RT-QuIC positive in 37/38(92.8%) cases tested. MRI brain demonstrated abnormalities associated with CJD in 60/64 cases (93.7%) cases (70% basal ganglia and cortex, 1.7% basal ganglia only, 28.3% cortex only). Cortical signal change contralateral to the side of affected limb occurred in 86.7%. In the isolated alien limb cases, 90.9% had contralateral parietal signal changes on MRI brain.

**Conclusion:** *Our large case series confirms that alien limb syndrome occurs in 6.7% of patients with CJD. The presence of this symptom complex in the context of a rapidly progressive neurological syndrome with cognitive decline should raise the suspicion of CJD.*

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### **P183 Characterization of 263K microsomal fraction PrP<sup>Sc</sup> assemblies and its relevance with regard to nanofiltration of plasma-derived products.**

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Following the emergence of variant Creutzfeldt-Jakob disease (vCJD) in the United Kingdom (UK) in 1996, four incidents of human-to-human transmission of vCJD prions in recipients of transfused, non-leukoreduced red blood (RBC) concentrates from donors with pre-clinical vCJD have been described in the UK, providing evidence that vCJD is transmissible through transfusion. With regards to plasma-derived medicinal products, there is no confirmed evidence so far that these products can transmit vCJD, most likely due to the efficacy of manufacturing processes of these products in removing prion-associated infectivity. Among steps susceptible to

reduce prion infectivity the efficacy of nanofiltration on 15, 20 or 35 nm pore size filters is debated with regards to uncertainties regarding the size of the agent that could potentially be present in plasma from vCJD incubating donors.

The evaluation of the prion removal efficacy of plasma product manufacturing process steps is carried out through spiking experiments in small-scale studies in laboratory setting. These studies measure the reduction of a prion load voluntarily added to the tested product for the purpose of the study. The infectivity present in blood (and plasma) in prion diseases is low and its physicochemical nature is still unknown. These studies therefore use brain material from experimentally infected animals, which exhibit high infectious titers. The brain material is either used directly as brain homogenates, or in a more enriched form such as microsomal fraction to eliminate the infectivity associated with the largest cellular debris.

Since the nature of the prion agent in blood is still undetermined today, the question of the relevance of the different spiking materials is still significant. To attempt to answer at least partly this question, the size and aggregation profiles of PrP<sup>Sc</sup> assemblies in 263K microsomal fractions were determined using velocity sedimentation gradients and was related to infectivity (Bioassay) or templating activity [quantitative miniaturized bead-PMCA –(mb-PMCA)] data in these same fractions. The results and analysis of these studies will be presented and discussed with regard to the efficacy of nanofiltration in eliminating small PrP<sup>Sc</sup> objects.

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#### **P184 Genes in prion protein conversion and in colorectal cells**

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Increasing evidence indicates that some genes may play a role in prion diseases and cancers, including colorectal cancer and hepatocellular carcinoma. Overexpression of cellular prion is observed in endothelial proliferation-to-differentiation switch of colorectal cancer cells. We had reported the differentially expressed genes that correlate with the prion degeneration using Affymetrix microarrays. Among 97 genes, the 85 were upregulated and 12 were downregulated as compared with negative controls. Gene Set Enrichment Analysis indicated that the differentially regulated genes were highly associated with cell growth and maintenance (*Amph*, *Slc40a1*, *Slpi*, *Mup5*, *Adm*, *Dpt*, and *Rsad2*), immune response (*Amph*, *Ly6f*, *Ly6c1*, *Ly6c2*, *Slc40a1*, and *Rsad2*) and cell-cell interaction system (*Gzme*, *Gzmd*, *Slpi*, *Adm*, and *Dpt*). Expression of these genes was examined in colorectal cancer cells. The identification of genes in both pathological states may provide an insight to the function of cellular prion in colorectal oncogenesis.

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#### **P185 Transmission of rare genetic prion disease with 6OPRI to humanized mice**

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Genetic prion diseases in humans are linked to mutations in the human PRNP gene. Point mutations in different codons cause distinct clinical and neuropathological manifestations ranging from those of a classical CJD to atypical profiles resembling those of GSS. Different prion variants, or strains, underlie this phenotypic diversity, which manifest as an ample variability in infective capability and biochemical properties.

Octapeptide repeat insertions (OPRI) occur in the octapeptide repeat region of the PRNP gene, spanning residues 51-91 of human PrP<sup>C</sup>, and are almost invariably associated with prion disease. Cases with different number of insertions have been found, the most frequent being 6OPRI.

Although symptoms and neuropathology show considerable variability between cases of 6OPRI, at the biochemical level they usually present a classical, three-band PrP<sup>res</sup> glycosylation pattern.

Transmissibility of 6OPRI prions have not yet been fully assessed using bioassay. In this study we report the transmission of an isolate of GSS 6OPRI to transgenic mice expressing the human PrP<sup>C</sup> 129M (Tg340 mice).

Humanized mice were intracerebrally inoculated with brain homogenate from a 39-year-old patient with clinical and neuropathological changes compatible with prion disease and a 144 bp insertion in the OPR region of the PRNP gene. The genetic, neuropathological and biochemical study of the case can be found in the corresponding case report (Xiao et al., 2013).

The isolate proved positive to PrP<sup>res</sup> on Western blot. The glycosylation pattern corresponded to a type 1 PrP<sup>res</sup>, with absence of low molecular weight bands.

Inoculated mice developed clinical signs suggestive of prion disease with a mean incubation period of 206±4 days post-inoculation, which is similar to that observed for animals inoculated with sCJD type 1. Clinical features consisted of ataxic gait with hypermetria, lethargy, weight loss, ruffled coat and kyphosis.

Neuropathologically the animals showed severe spongiform degeneration in frontal and thalamic cortex and in the Ammon's horn of the hippocampus, while the dentate gyrus was unaffected, a feature termed "hippocampal signature". The diencephalon and the brainstem were relatively spared, and the cerebellum was poorly affected. On IHC a coarse granular pattern was observed in frontal and thalamic cortex, hippocampus, septal area, thalamus and hypothalamus, while immunostaining in caudal areas consisted of a widely distributed punctuate.

On Western blot, a PrP<sup>res</sup> type 1 was found, identical to that of the original inoculum.

In conclusion, although cases of prion disease with 6OPRI have previously proved to be transmissible to humanized models, no in-depth histopathological or biochemical assessment of experimentally infected mice has been done to date. Our results indicate that, in agreement with the finding of classical glycosylation patterns in human patients, the transmission characteristics of these isolates are similar to those of sCJD.

**P186 Sporadic Creutzfeldt-Jakob Disease in a Woman Married into a Gerstmann-Sträussler-Scheinker Family: An Investigation of Prions' Transmission via Microchimerism**

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This is the first report of presumed sporadic Creutzfeldt-Jakob disease (sCJD) and Gerstmann-Sträussler-Scheinker disease (GSS) with the prion protein gene (*PRNP*) c.305C>T mutation (p.P102L) occurring in one family, which had the father and son with GSS and the mother with a rapidly progressive form of CJD. Diagnosis of genetic, variant, and iatrogenic CJD was ruled out based on the mother's clinical history, genetic tests, and biochemical investigations, all of which supported the diagnosis of sCJD. However, given the low incidence of sCJD and GSS, their co-occurrence in one family is extraordinary and challenging. Thus, a hypothesis for the transmission of infectious prion proteins (PrP<sup>Sc</sup>) via microchimerism was proposed and investigated. DNA from 15 different brain regions and plasma samples of the CJD patient was subjected to PCR and shallow sequencing for detection of a male sex-determining chromosome Y (chr.Y). However, no trace of chr.Y was found. A long CJD incubation period or presumed small concentrations of chr.Y may explain the obtained results. Further studies of CJD and GSS animal models, with controlled genetic and proteomic features, are needed to conclude whether maternal CJD triggered via microchimerism by a GSS fetus might present a new PrP<sup>Sc</sup> transmission route.

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**P187 Diagnosis of Methionine/Valine Variant Creutzfeldt - Jakob disease using PMCA in Cerebrospinal fluid**

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**Background:**

Variant Creutzfeldt-Jakob disease (vCJD) is a rare human prion disease caused by infection with a bovine prion. Although all clinical cases of vCJD evaluated for the prion protein gene, *PRNP*, have been methionine homozygotes at codon 129 for 20 years, the first definite heterozygous vCJD patient, with Methionine/ valine (MV) genotype, was recently described in the United Kingdom. This patient met the diagnostic criteria for probable sporadic CJD (sCJD) but neuropathology and molecular analysis of prion strain type at autopsy confirmed vCJD. This recent publication underlined previous concern about a possible second wave of vCJD cases attributed to alternative genotype at codon 129 and secondary transmission. Iatrogenic

transmission of vCJD by blood transfusion has already been documented in three recipients of non-leucodepleted red blood cell concentrates from donors in incubation of the disease. One additional probable case of vCJD transmission by blood transfusion was revealed at autopsy in a MV patient who died from a non-neurological disorder when vCJD prion protein was detected in his spleen. A highly sensitive and specific pre-mortem test to discriminate MV vCJD from sCJD is urgently needed to rapidly and accurately diagnose any future MV vCJD patients.

#### Methods:

We adapted our ultrasensitive blood detection assay based on protein misfolding cyclic amplification (PMCA) technology for the specific detection of vCJD in the cerebrospinal fluid (CSF) to identify vCJD infected individuals including the recent MV patient.

#### Results:

Among the 98 CSF samples analysed blinded to the diagnosis, our assay identified specifically 40 of the 41 vCJD samples thus achieving a diagnostic sensitivity of 97.6%. Importantly, our assay allows us to discriminate the heterozygous MV vCJD case from the 12 MV neuropathologically confirmed sCJD patients tested. In addition, the PrP<sup>TSE</sup> molecular signature obtained after amplification of the CSF from the MV vCJD patient was similar to those obtained MM vCJD patients (Type2B). Our assay also showed 100% analytical specificity, with none of the 57 potentially cross-reacting CSF specimens from patients with sCJD, genetic CJD, Alzheimer's disease, and other non-neurodegenerative diseases giving a positive result.

#### Conclusions:

These results indicate that CSF PMCA may allow the identification of MV vCJD *in life* and, in combination with other tests such as CSF real-time quaking-induced conversion, accurate discrimination from sCJD.

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### **P188 Age at infection and PRNP codon 129 genotype are associated with susceptibility to vCJD in gene targeted mouse models expressing human PrP**

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The retrospective Appendix studies have indicated that up to 1 in 2000 individuals across all three *PRNP* codon 129 genotypes and in different age cohorts have evidence of abnormal PrP. This is in stark contrast to the actual number of vCJD cases which have occurred and could indicate the potential for a long subclinical phase of disease. Currently the majority of our knowledge of vCJD pathogenesis is limited to post mortem examination of cases of vCJD or endpoint mouse studies. We have now undertaken a series of time course studies in young, adult and aged gene targeted mice expressing each codon 129 genotype in order to model the impact of age and codon 129 genotype on vCJD pathogenesis.

Gene targeted mice expressing each codon 129 genotype, referred to as HuM, HuMV and HuV, have been intracerebrally inoculated with vCJD at 20 (young), 100 (adult) and 300 (aged) days old. Mice were culled at 200, 300 (aged animals only), 400, and 500 and 600 (young and adult animals only) days post inoculation and tissues harvested. Tissues were also collected from any intercurrent deaths where possible. Initial studies were carried out assessing vacuolation (H&E), presence of plaques (H&E) and PrP deposition (antibody 3F4) in the brain.

Susceptibility to vCJD decreased in the order HuM>HuMV>HuV with only HuM mice showing evidence of both vacuolation and plaques (H&E) from 500dpi. All three genotypes had evidence of PrP deposition with the numbers of mice affected and the timing of PrP deposition influenced by genotype. Higher numbers and the first appearance of deposition in HuM mice at 200dpi whereas in HuMV and HuV it was first observed at approximately 400dpi in lower numbers.

We demonstrate that age of infection impacts on vCJD susceptibility and pathogenesis. Aged mice were less likely to demonstrate evidence of disease overall. No vacuolation or plaques were observed and PrP deposition was only observed in HuM and HuMV mice. Although young and adult mice had evidence of disease at similar time points in all three genotypes (as above), the proportions of mice affected at each timepoint varied with age of inoculation; this was more apparent in the HuM mice and requires further analysis.

We provide a systematic comparison of vCJD pathogenesis in gene targeted mice inoculated at a range of ages. We have generated experimental evidence that susceptibility to disease is not only associated with codon 129 genotype but also with age of infection. In addition, we highlight that neuropathological characteristics can vary according to both genotype and the age at time of infection. Further studies will include biochemical characterisation and comparison of peripheral infectivity. These datasets add to our experimental knowledge of vCJD pathogenesis allowing the further development of risk assessments and risk reduction measures.

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#### **P189 Critical revision of diagnostic utility of 14-3-3 and RT-QuIC assay in Creutzfeldt-Jakob disease diagnosis**

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Early and accurate in vivo diagnosis of Creutzfeldt-Jakob disease (CJD) is necessary for quickly distinguishing treatable from untreatable rapidly progressive dementias and for future therapeutic trials.

Recent revisions of diagnostic criteria for sCJD diagnosis included real-time quaking-induced conversion (RT-QuIC) seeding assay in the cerebrospinal fluid (CSF) in addition to 14-3-3. RT-QuIC is a new in vitro prion amplification technology that can detect minute amounts of the disease-specific pathologic prion protein.

Aim of our study is to compare the diagnostic utility of 14-3-3 testing and RT-QuIC assay.

We collected CSF samples from 1040 patients with rapidly progressive dementia sent to our Neuropathology Laboratory for CJD diagnosis from January 1st 2015 to December 31st 2017.

CSF samples were tested for 14-3-3 positivity by Western blot and by RT-QuIC assay. Total tau protein concentration was also determined as supporting biomarker with a diagnostic cut-off of 1300 pg/ml for sCJD diagnosis.

69 subjects were then diagnosed as definite sCJD. 204 out of 1040 CSF samples were 14-3-3 positive and 128 showed tau levels >1300 pg/ml. RT-QuIC testing was positive in 67 out of 69 CSF samples of definite sCJD subjects; the two RT-QuIC negative subjects underwent to olfactory mucosa brushing, were then tested by RT-QuIC and were finally found positive. Among sCJD, 5 cases had a negative 14-3-3 and 11 cases showed tau levels <1300 pg/ml.

70 out of 128 non sCJD cases showed tau >1300 pg/ml. At follow-up patients with a positive 14-3-3 and negative RT-QuIC testing were diagnosed as other dementias or other neurological disorders.

14-3-3 was the first biomarker included in diagnostic criteria for sCJD since 1998. 14-3-3 is a surrogate biomarker and represents a neuronal degeneration marker. RT-QuIC assay can specifically identify disease-specific pathologic prion protein. In fact we confirmed 100% sensitivity and specificity for RT-QuIC assay. 14-3-3 testing showed 93% sensitivity and 86% specificity, while tau protein quantification, with a 84% sensitivity had a 93% specificity.

The present study confirms that RT-QuIC is a 100% sensitive and 100 specific assay for diagnosing sCJD, as we previously reported (1). Olfactory mucosa brushing is mandatory when CSF is negative.

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### **P190 Human prion disease mortality rates by occurrence of chronic wasting disease in free-ranging cervids, United States**

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#### Background

Chronic wasting disease (CWD) is a prion disease of deer and elk that has been identified in free-ranging cervids in 23 US states. While there is currently no epidemiological evidence for zoonotic transmission through the consumption of contaminated venison, studies suggest the CWD agent can cross the species barrier in experimental models designed to closely mimic humans. We compared rates of human prion disease in states with and without CWD to examine the possibility of undetermined zoonotic transmission.

#### Methods

Death records from the National Center for Health Statistics, case records from the National Prion Disease Pathology Surveillance Center, and additional state case reports were combined to create a database of human prion disease cases from 2003-2015. Identification of CWD in each state was determined through reports of positive CWD tests by state wildlife agencies. Age- and race-adjusted mortality rates for human prion disease, excluding cases with known etiology, were determined for four categories of states based on CWD occurrence: highly endemic (>16 counties with CWD identified in free-ranging cervids); moderately endemic (3-10 counties with CWD); low endemic (1-2 counties with CWD); and no CWD states. States were counted as having no CWD until the year CWD was first identified. Analyses stratified by age, sex, and time period were also conducted to focus on subgroups for which zoonotic transmission would be more likely to be detected: cases <55 years old, male sex, and the latter half of the study (2010-2015).

#### Results

Highly endemic states had a higher rate of prion disease mortality compared to non-CWD states (rate ratio [RR]: 1.12, 95% confidence interval [CI] = 1.01 - 1.23), as did low endemic states (RR: 1.15, 95% CI = 1.04 - 1.27). Moderately endemic states did not have an elevated mortality rate (RR: 1.05, 95% CI = 0.93 - 1.17). In age-stratified analyses, prion disease mortality rates among

the <55 year old population were elevated for moderately endemic states (RR: 1.57, 95% CI = 1.10 – 2.24) while mortality rates were elevated among those ≥55 for highly endemic states (RR: 1.13, 95% CI = 1.02 - 1.26) and low endemic states (RR: 1.16, 95% CI = 1.04 - 1.29). In other stratified analyses, prion disease mortality rates for males were only elevated for low endemic states (RR: 1.27, 95% CI = 1.10 - 1.48), and none of the categories of CWD-endemic states had elevated mortality rates for the latter time period (2010-2015).

### Conclusions

While higher prion disease mortality rates in certain categories of states with CWD in free-ranging cervids were noted, additional stratified analyses did not reveal markedly elevated rates for potentially sensitive subgroups that would be suggestive of zoonotic transmission. Unknown confounding factors or other biases may explain state-by-state differences in prion disease mortality.

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### **P191 A rapid endogenous model for prion removal evaluation from whole blood and plasma**

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Prion diseases or Transmissible Spongiform Encephalopathies (TSEs) are neurodegenerative diseases including the variant of Creutzfeldt-Jakob disease (vCJD) in humans. It is now evident that vCJD can be transmitted from person to person through blood transfusion and that this infectious agent is present in the blood of affected individuals long before first symptoms occur. The publication, in 2017, of the first definite heterozygous methionine/valine vCJD patients emphasizes previous concerns regarding a possible second wave of vCJD cases. In the UK, 1/2000 subjects would be potentially infected by vCJD, justifying the need to develop some processes for prion removal in blood products. Here we propose a new endogenous model based on the international standard hamster 263K model for the evaluation of prion removal from whole blood and plasma.

**Methods:** In this study, groups of healthy hamsters (8 animals per group) were inoculated intraperitoneally with hamsters blood products (whole blood and plasma) collected in sick animals at the terminal stage of the disease (500 µL or 1 mL). Animals were observed until the occurrence of clinical signs and the presence of abnormal prion protein PrP<sup>TSE</sup> was determined by western blot in the brain of animal after culling.

**Results:** Whole blood and plasma from affected animals transmitted the disease with 100% efficacy. The mean survival time for hamster groups infected with 1 mL of total blood or plasma was 222 days and 237 days respectively. Histological and biochemical analyzes showed that sick animals had prion disease with presence of abnormal prion protein deposits, associated with spongiosis and astrogliosis.



## **P192 Prion amplification techniques for the rapid evaluation of surface decontamination procedures**

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### Aims:

Transmissible Spongiform Encephalopathies (TSE) or prion diseases are a group of incurable and always fatal neurodegenerative disorders including Creutzfeldt-Jakob diseases (CJD) in humans. These pathologies include sporadic (sCJD), genetic and acquired (variant CJD) forms. By the past, sCJD and vCJD were transmitted by different prion contaminated biological materials to patients resulting in more than 400 iatrogenic cases (iCJD). The atypical nature and the biochemical properties of the infectious agent, formed by abnormal prion protein or PrP<sup>TSE</sup>, make it particularly resistant to conventional decontamination procedures. In addition, PrP<sup>TSE</sup> is widely distributed throughout the organism before clinical onset in vCJD and can also be detected in some peripheral tissues in sporadic CJD. Risk of iatrogenic transmission of CJD by contaminated medical device remains thus a concern for healthcare facilities. Bioassay is the gold standard method to evaluate the efficacy of prion decontamination procedures but is time-consuming and expensive. Here, we propose to compare *in vitro* prion amplification techniques: Protein Misfolding Cyclic Amplification (PMCA) and Real-Time Quaking Induced Conversion (RT-QuIC) for the detection of residual prions on surface after decontamination.

### Methods:

Stainless steel wires, by mimicking the surface of surgical instruments, were proposed as a carrier model of prions for inactivation studies. To determine the sensitivity of the two amplification techniques on wires (Surf-PMCA and Surf-QuIC), steel wires were therefore contaminated with serial dilutions of brain homogenates (BH) from a 263k infected hamster and from a patient with sCJD (MM1 subtype). We then compared the different standard decontamination procedures including partially and fully efficient treatments by detecting the residual seeding activity on 263K and sCJD contaminated wires. We completed our study by the evaluation of marketed reagents endorsed for prion decontamination.

### Results:

The two amplification techniques can detect minute quantities of PrP<sup>TSE</sup> adsorbed onto a single wire. 8/8 wires contaminated with a 10<sup>-6</sup> dilution of 263k BH and 1/6 with the 10<sup>-8</sup> dilution are positive with Surf-PMCA. Similar performances were obtained with Surf-QuIC on 263K: 10/16 wires contaminated with 10<sup>-6</sup> dilution and 1/8 wires contaminated with 10<sup>-8</sup> dilution are positive. Regarding the human sCJD-MM1 prion, Surf-QuIC allows us to detect 16/16 wires contaminated with 10<sup>-6</sup> dilutions and 14/16 with 10<sup>-7</sup>. Results obtained after decontamination treatments are very similar between 263K and sCJD prions. Efficiency of marketed treatments to remove prions is lower than expected.

### Conclusions:

Surf-PMCA and Surf-QuIC are very sensitive methods for the detection of prions on wires and could be applied to prion decontamination studies for rapid evaluation of new treatments. Sodium hypochlorite is the only product to efficiently remove seeding activity of both 263K and sCJD prions.

**P193 Optimisation of codon 129 genotyping from paraffin-embedded formalin fixed tissue using restriction fragment length polymorphism analysis**

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A predisposition to Creutzfeldt-Jakob disease (CJD) has been shown to be associated with the polymorphism at codon 129 of the prion protein gene (*PRNP*). Codon 129 genotyping can therefore aid in the identification of genetic forms of the disease and is an essential component in the sub-classification of human prion disease.

*PRNP* codon 129 genotyping is routinely carried out from fresh/frozen tissues using molecular techniques including DNA extraction, polymerase chain reaction (PCR) and restriction fragment length polymorphism analysis (RFLP). However, in cases in which only formalin fixed paraffin embedded (FFPE) tissue is available, *PRNP* codon 129 genotyping is much more challenging. The quality and quantity of nucleic acid extracted from FFPE tissues are significantly reduced as formalin causes the fragmentation of DNA and cross-linking between DNA and protein, lowering the success of PCR amplification. Many studies have been carried out to try and resolve these complexities, however it remains a significant problem where only FFPE tissue is available.

This work evaluates the complexities of using these techniques, with the specific aim of genotyping the *PRNP* codon 129 variant from formalin-fixed tissue, FFPE tissue, and formic-acid treated FFPE for CJD studies.

This comparative analysis involved (i) Evaluation of two DNA extraction methods (phenol chloroform and silica-based commercial kits, NucleoSpin® DNA FFPE XS and QIAamp DNA FFPE Tissue), (ii) Quantification of the DNA using PicoGreen® dsDNA assay kit, (iii) Qualification of the integrity of the DNA by comparing multiple primer combinations for PCR amplification, (iv) and RFLP enzyme digestion of PCR amplicons to determine the codon 129 genotype. Formalin-fixed tissue from the Frontal Cortex and the Cerebellum from non-CJD cases were selected. The amplification of the extracted DNA was carried out by conventional PCR using specific primers for a 576bp and a 177bp region of *PRNP* containing the polymorphic sequence for the codon 129 residue. These products were digested with the restriction enzyme *NspI*.

Findings show that the phenol-chloroform method showed the highest concentration of DNA extracted, however silica-based kits were equally successful in downstream processing. Optimisation of the PCR and RFLP conditions are underway, as currently the results are not consistently reproducible.

Assay difficulties may arise due to PCR-introduced artefacts which are known to occur as a side effect of formalin fixation or the restriction enzyme digest not running to completion. Although PCR amplification appears successful, knowing artificial alterations can be present, further studies and optimisation of the techniques need to be pursued before we can take these conditions forward for diagnostic testing of FFPE tissues, and formic-acid treated FFPE.

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**P194 A rapid and highly sensitive amplification method for the detection of prions in cerebrospinal fluid of variant Creutzfeldt-Jakob disease patients**

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Human prion diseases are a group of neurodegenerative conditions associated with misfolding and aggregation of the prion protein. These disorders are invariably fatal, and they are classified according to their clinical, neuropathological and molecular characteristics as sporadic, genetic, or acquired, with neuropathological variations in each group.

Variant Creutzfeldt–Jakob disease (vCJD) is an acquired and zoonotic form of human prion disease linked to oral exposure to the infectious agent that causes bovine spongiform encephalopathy. Currently, 178 confirmed cases of vCJD have been recognized in the United Kingdom. Genetic analysis has shown that nearly all reported cases had methionine homozygosity (methionine/ methionine or MM) at codon 129 of the human *PRNP* gene. The identification in 2017 of the first pathologically confirmed case of vCJD in a heterozygous individual (methionine/valine or MV), underlines public health concerns about the possibility of additional cases of vCJD in *PRNP* codon 129 MV and VV individuals and the potential for secondary transmissions e.g. via blood transfusion.

The development of seeded conversion assays, such as Protein Misfolding Cyclic Amplification (PMCA) and Real-Time Quaking-Induced Conversion (RT-QuIC), has the potential to simplify the clinical diagnostic of Creutzfeldt–Jakob disease (CJD) and other protein misfolding disorders of the central nervous system. Rapidity, high sensitivity and specificity, and minimal manipulation of clinically available specimens are all crucial aspects to consider in the development of such diagnostic tests for human prion diseases.

We report a rapid and highly sensitive seeded conversion assay, based on the *in vitro* amplification method PMCA. Our highly sensitive amplification assay, hsPMCA, was able to detect the presence of the disease-associated prion protein in cerebrospinal fluid (CSF) from 15 out of 15 definite, probable and possible vCJD cases, all collected during the symptomatic phase of the illness. Further evaluation using a blinded panel of CSF samples from vCJD, sporadic CJD, other neurodegenerative disease, and control patients, showed an estimated sensitivity and specificity for the assay of 100% for vCJD. Importantly hsPMCA was able to detect the diseases associated form of the prion protein rapidly, with minimal manipulation of the clinical specimen, and notably, using CSF from vCJD patients who were either MM or MV at *PRNP* codon 129. These findings are a significant advance in the development of a pre-mortem diagnostic test for vCJD and differential diagnosis of CJD as a whole.

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#### **P195 Event-based model of Diffusion MRI shows that sCJD strains have similar epicenter but different lesion propagation in the brain**

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Sporadic Creutzfeldt-Jakob disease (sCJD) has different clinico-pathological features based on a molecular classification in 5 strains (subtypes) that depend on the polymorphism (methionine, M, or valine, V) at codon 129 and on two types of the misfolded prion protein. Establishing in vivo biomarkers enabling the prediction of sCJD subtype is very relevant for patient management and clinical trials. MRI is an ideal candidate for delivering such biomarker, because it illustrates in vivo the distribution of the brain lesions for each sCJD patient. To date, studies dealing with the evolution of DWI signal abnormalities as a function of disease progression are few, and are all limited by the lack of disease subtype separation and the use of small cohort.

The aim of this study was to model the DWI signal abnormalities evolution in 5 sCJD strains in order to determine the starting point (i.e. epicenter) and the direction of propagation of the DWI signal abnormalities in the brain.

A neuroradiologist (AB) blind to the pathological diagnosis visually scored the DWI of 306 patients with definite autopsy diagnosis of sCJD subtype and 123 patients with ruled-out diagnosis of prion disease. Patients with 5 sCJD pure subtypes were included: MM/MV1 (n=89), MM/MV2C (n=42), MV2K (n=22), VV1 (n=15) and VV2 (n=49). A semi-quantitative method based on a four-point (0-3) ordinal integer scale was implemented to visually score the images and grade the hyperintensities of the DWI in 12 specific brain regions.

To assess the DWI signal progression in each sCJD subtype, we considered a novel data-driven model, the event-based model (EBM), recently introduced to study the evolution of Alzheimer's and Huntington's diseases. The EBM provides a description of the disease progression in terms of a sequence of events from a cross-sectional dataset. An event is defined as the switching from a normal to an abnormal state for a biomarker of a patient. In our case, we considered a set of 12 events related to the 12 brain region scores used to assess the diffusion images. The EBM finds the most likely sequence of DWI abnormalities in each sCJD subtype given the observed DWI measurements of the subjects.

The main result of this study showed that the 5 different strains have different sequence of lesion propagation. The anterior cingulate cortex was affected very early in all strains. Then, three patterns emerged from the orderings: neocortex affected before striatum in MM/V1 and MM/V2C; striatum affected before neocortex in VV2 and MV2K; limbic regions affected before neocortex and striatum in VV1.

In conclusion, EBM provided for the first time data-driven models of evolution of DWI signal hyperintensities in sCJD subtypes: the characteristic orderings are based on a cross-sectional dataset and their longitudinal consistency has been validated comparing the stages at follow-ups with the baseline.

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#### **P196 PMCA-replicated PrP<sup>Sc</sup> in urine of vCJD patients maintains strain characteristics of brain PrP<sup>Sc</sup>: Transmission study**

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Reliable demonstration of PrP<sup>Sc</sup> in cerebrospinal fluids by amplification technology has been achieved providing a reliable diagnosis of prion diseases. Similar diagnostic tests based on other more easily obtainable body fluids are under study. However, the properties of the PrP<sup>Sc</sup> present in body fluids, such as infectivity and strain characteristics, remain undefined. The presence of PrP<sup>Sc</sup> in urine of vCJD patients has been recently demonstrated by protein misfolding cyclic amplification (PMCA)<sup>1</sup>. We have now tested transmission properties of the amplified vCJD-urine PrP<sup>Sc</sup> by bioassay in transgenic (Tg) mice expressing human PrP<sup>C</sup>-129M. We also inoculated 96 Tg mice with raw vCJD-urine to test the level of infectivity in unamplified urine. Successful transmission of PMCA-treated urine PrP<sup>Sc</sup> was characterized by an attack rate of 100% and an incubation period (days post inoculation, dpi) of 661±45 and 713±36 depending on the final resuspension concentration, 10x and 1x of the original urine-volume, respectively. Incubation times slightly exceeded those obtained with vCJD-brain homogenate (BH): dpi 574±104 and 648±39 when BH 10% and 1% was inoculated respectively. The histopathological features of the condition transmitted by PMCA-treated urine were similar to those obtained with BH as for occurrence and distribution of spongiform degeneration and florid plaques. Furthermore, western blot profile of the PK-resistant PrP was type 2B as observed in the vCJD-BH as well as in vCJD-inoculated mice and in PMCA-treated urine. None of the 95 mice inoculated with raw vCJD-urine resulted positive for prion disease up to 707 dpi. This result is not surprising considering the minute amount of PrP<sup>Sc</sup> present in urine, which has been calculated to be 3×10<sup>-21</sup> mol. per milliliter by quantitative PMCA<sup>1</sup>. Lack of transmissibility was also observed, in the same Tg mice line, with urine from sCJD-MM1<sup>2</sup>. These findings indicate that in vCJD, urine excreted PrP<sup>Sc</sup>, although minimal in amount, maintains infectivity and strain characteristics of the PrP<sup>Sc</sup> present in brain.

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2. *Notari S, Qing L, Pocchiari M, et al. Assessing prion infectivity of human urine in sporadic Creutzfeldt-Jakob disease. Emerg Infect Dis. 2012; 18:21-8.*

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### **P197 Subtype diagnosis in Sporadic Creutzfeldt-Jakob Disease with Diffusion Magnetic Resonance Imaging: a study of 429 Patients with Definite Pathology**

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Early diagnosis is critical to achieving an effective treatment of prion diseases. Accurate subtype diagnosis is particularly important in sporadic Creutzfeldt-Jakob disease (sCJD) that include distinct prion strains (MM/MV1, MM/MV2C, MV2K, VV1, VV2) that may respond differently to drug treatment. Diagnostic accuracy of MRI with diffusion-weighted imaging (DWI) in early

diagnosis of sCJD has been reported to be above 90%. However, so far the diagnosis of sCJD subtype has not been comprehensively explored with DWI.

The two aims of this study are i) to measure DWI diagnostic reliability and compare it with that of CSF tests (i.e., 14-3-3 and tau); ii) build an algorithm to diagnose sCJD subtypes using the MRI lesion profile obtained by DWI.

A cohort of 306 sCJD patients with autopsy confirmed subtype diagnosis were provided by NPDPS. A group of 123 patients with prion diagnosis ruled out acted as control. A neuroradiologist (AB) blind to the diagnosis scored the DWI of the 429 patients. A semi-quantitative method based on a four-point (0-3) ordinal scale was implemented to grade the hyperintensities of the DWI in 12 specific brain regions. To assess the inter-rater reliability (IRR) of the scoring system, other two neuroradiologists (MG, RL) blind to the final diagnosis scored the DWI of 150 subjects.

First, we measured the DWI diagnostic reliability in 429 patients, then we compared it with those of CSF tests in a subset of 215 patients (170 sCJD and 45 non prion) that had undergone MRI, 14-3-3 and tau tests. The overall diagnostic reliability of DWI in 429 patients was 91% sensitivity and 98% specificity, with excellent IRR among the three neuroradiologists in most brain regions. Furthermore, in the subgroup of 215 patients the sensitivity and specificity of DWI (94% and 94%) surpassed those of 14-3-3 (76% and 87%) and tau (80% and 84%).

Second, to predict the sCJD subtype with the MRI lesion profile, we adopted an algorithm in the form of a decision tree. This algorithm produced 9 distinct MRI phenotypes that characterized the five sCJD pure subtypes. The accuracy in predicting the correct subtype was about 62% and increased to 90% if the codon 129 polymorphism was considered. Key steps in the algorithm are the presence of DWI hyperintensity in the neocortex, striatum, thalamus and cerebellum.

This work confirmed the excellent diagnostic reliability of MRI for the early diagnosis of sCJD. The study also provides the neurologists with new diagnostic tools for subtype identification at the patient's bedside.

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#### **P198 New PMCA-based HTS system that uses recombinant infectious prions to detect anti-prion compounds**

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Currently, no treatment exists for the Transmissible Spongiform Encephalopathies or prionopathies despite the continuous efforts of the scientific community. Different strategies have been proposed to deal with these devastating disorders characterized by the misfolding of PrP<sup>C</sup> into PrP<sup>Sc</sup> and the accumulation of amyloid fibrils in the Central Nervous System: 1) boosting the immunological response, 2) reducing the expression of PrP<sup>C</sup>, 3) inhibiting the pathway that leads to neuronal death, 4) increasing the capacity of protein aggregate elimination and 5) introducing distinct molecules with the ability to hinder the misfolding of PrP<sup>C</sup>, among others. The lack of

knowledge about the molecular mechanisms of these diseases makes the screening for compounds to inhibit prion propagation one of the most straightforward and promising approaches. Testing a significant number of compounds requires a fast, robust and easily implementable system. *In vitro* prion propagation techniques based on recombinant PrP fulfilling most of these requirements.

In order to establish a high-throughput screening (HTS) system for the detection of chemical compounds with anti-prion activity, we have developed a new methodology based on the well-known Protein Misfolding Cyclic Amplification (PMCA). In the novel system presented herein, we changed the sonication/incubation cycles characteristic of PMCA for shaking as the source of energy. Moreover, a simplified substrate devoid of brain factors composed by full-length bank vole recPrP and other cofactors is used, making the technique very robust and easily adaptable to a HTS format. This single cofactor-complemented substrate allows the measurement of the presence of *bona fide* misfolded proteins using Thioflavin T (ThT) in a plate reader. Since shaking devices are simpler and less costly than sonicators, the system is easily scalable and implementable in any laboratory. Another important feature of the new method is the possibility of working with faithful recombinant infectious prions obtained *in vitro*, what allows the detection of the possible anti-prion compounds in a real infectious prion formation process in contrast to other techniques based on recombinant PrP fibrillization. Altogether, this new HTS method allows us to test 50-100 compounds per day with the possibility to observe direct inhibition of *bona fide* prion propagation.

Here we present the HTS of a commercially available library of 2,500 small chemical compounds using this newly developed system. From the library tested, we obtained two promising hits as final result, L2.JM33 and L2.JM56, demonstrating the utility of the method for the high-throughput screening of big chemical compound libraries.

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#### **P199 New Strategies for Prion Diseases: Drugs for Stabilization of Globular Domain of the Cellular Prion Protein interacting in the Copper(II) Binding Region**

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The availability of high-resolution structures of PrPC (obtained by NMR and X-ray crystallography) allows the potential identification of novel PrPC ligands by employing computer-based methodologies. The globular domain of PrPC has been scouted to define solvent-accessible pockets that could be targeted with small molecules. To achieve this goal, two recently described, complementary methods have been employed: (i) fpocket, an empirical high-throughput method, and (ii) MDmix, which is physics-based and more reliable, but with a lower throughput. Combined, these methods will be applied to a large set of target candidates to obtain good confidence on positive predictions. This method also reveals the interactions preferences of the protein and identifies non-displaceable water molecules. Preliminary studies, performed using human PrP (1QLZ.pdb) and vole PrP (2K56.pdb), suggest the presence of a druggable region around His187 residue. Importantly this region corresponds a copper(II) binding region that is essential for the aggregation of native PrP in its infectious form. The prion protein (PrP) binds copper that, under some conditions, can facilitate its folding into a more protease resistant form. Hence, copper levels may influence the infectivity of the scrapie form of prion protein (PrPSc). Our research team is intensely involved in the design of small peptides as potential biocompatible

agents to remove copper(II) ions from senile plaques (Alzheimer's disease; AD). We have recently demonstrated that several tripeptides have great potential for the design/development of potential anti-Alzheimer drugs and/or diagnostic agents showing that tripeptides of the type HXaaH (namely histidine-any aminoacid-histidine) have a strong affinity for copper(II) ions (through the formation of a highly stable ATCUN motif). These copper chelators oligopeptides have been tested in the PrP model obtaining promising results in the preliminary in-vitro tests.

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## **P200 Towards optimal clinical trial design in sporadic CJD**

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Clinical trials in sporadic Creutzfeldt-Jakob Disease (sCJD) require meaningful endpoints beyond survival; ideally involving easily-obtained measures of routine clinical data, directly relevant to patients. A major advance has been the introduction of the MRC Scale, a composite, functionally-oriented endpoint recordable via telemedicine. Given the rapidly progressive, universally terminal course of sCJD, placebo-controlled trials may not be appropriate. Well-powered natural history datasets with a range of clinical endpoints are a practical alternative.

We sought to develop supplementary outcome measures to complement the MRC Scale in a clinical trial setting, using robust natural history data prospectively collected in the National Prion Monitoring Cohort. Cognitive and motor dysfunction occurs inevitably in sCJD patients, and can often be objectively identified during routine bedside clinical assessment.

### Methods & Results

We used item response modelling (IRT) to develop rating scales for motor and cognitive portions of the bedside neurological examination. Initial scales were derived using a subset of complete sCJD patient bedside assessments (motor n=148, cognitive n=230), based on fit to the IRT model. Simple linear regression was applied to the scales in a wider cohort of sCJD patients, each with 2 longitudinal assessments (motor scale n=124, cognitive scale patients n=84).

The final two scales are each scored 0-20, and show good fit to the IRT model ( $\chi^2 < 30$ ,  $p < 0.05$ ). The motor scale (motscale) includes tests of extrapyramidal and cerebellar dysfunction, power, eye movements, gait and speech; the cognitive scale (cogscale) includes executive function, episodic memory, language, attention, visual perception and calculation. Each scale can be completed in less than 10 minutes.

Both scales correlated with the MRC Scale (cogscale  $r=0.50$ , motscale  $r=0.85$ ), and had good standardised effect sizes longitudinally (cogscale Cohen's  $d=1.11$ , mean inter-assessment days=44.38, 95% CI 36.0-52.8; motscale Cohen's  $d=0.85$ , mean inter-assessment days=41.7, 95% CI 36.7-46.7). Inter-rater correlation was acceptable for both (motscale Cohen's kappa=0.58, cogscale=0.48). Both scales longitudinally demonstrated a linear decline over days ( $p < 0.05$ ). In this mixed codon 129 sCJD patient group, motor scale score was reduced by a mean 10% every 31.2 days (95% CI, 25.6-40.0), and cognitive scale by 10% every 18.5 days (95% CI, 14.9-24.3). This compares with a 10% decline in the MRC Scale every 5.3-27.8 days, depending on codon 129 genotype.



## Conclusions

We developed two clinical rating scales for different aspects of the routine clinical examination of sCJD patients. Both correlate with the MRC Scale and have moderate effect sizes longitudinally over 2 clinical assessments. Future work will extend the regression model to all longitudinal assessments, explore effects of codon 129 and other predictors of decline, and simulate use in future clinical trials for sCJD.

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## **P201 GWAS in sCJD: identification, validation and replication of 3 novel non-*PRNP* susceptibility loci**

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Sporadic Creutzfeldt-Jakob disease (sCJD) is the most common human prion disease, in which the cellular prion protein (PrP<sup>C</sup>) appears to spontaneously misfold and aggregate into a disease-associated form leading to invariably fatal neurodegeneration. Although there is clear genetic control in sCJD, no loci despite *PRNP* have previously been identified as consistently significant. However, through worldwide collaboration, a genome wide association study (GWAS) recently performed by our group analysing 4110 cases and 13569 control individuals has identified at least three new loci in addition to *PRNP*, in or near to *STX6*, *GAL3ST1* and *PDIA4*, which confer risk at genome-wide significance.

Here we present ongoing validation, replication and investigation of the biological relevance of these novel findings. First, validation was performed by evaluating allele clustering of top hits and genotyping of imputed SNPs using a second assay. We are now doing replication using hundreds of additional cases compared to thousands of new control samples, using qPCR genotyping assays for SNPs rs3747957, rs2267161 and rs9065 respectively. We are generating knock-out N2aPK1 cells for each candidate gene in order to challenge them in automated scrapie cell assays.

Each of these three loci has potential to provide key insights into the biology of prion disease. For example, as a component of the endocytic and secretory pathways, the role *STX6* could play in both PrP<sup>C</sup> misfolding and trafficking is clear. As a known GWAS hit for the tauopathy Progressive Supranuclear Palsy, as well as an eQTL with increased expression of the risk allele in the putamen, this protein has strong biological relevance as a risk factor for sCJD.

All GWAS results should be anchored in replication evidence, but functional and expression studies also strengthen GWAS findings and evaluate their relevance for the disease pathophysiology.

*Cover image: Raxoi Palace & cathedral  
Foto Turismo de Santiago*