Prion

ISSN: 1933-6896 (Print) 1933-690X (Online) Journal homepage: https://www.tandfonline.com/loi/kprn20

Prion 2016 Poster Abstracts

To cite this article: (2016) Prion 2016 Poster Abstracts, Prion, 10:sup1, S37-S127, DOI: 10.1080/19336896.2016.1162644

To link to this article: https://doi.org/10.1080/19336896.2016.1162644

Prion, 10:S37–S127, 2016
ISSN: 1933-6896 print / 1933-690X online

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P-001: Employing dynamic mass redistribution to identify pharmacological chaperones for the cellular prion protein

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The key pathogenic event underlying all forms of prion diseases is the conversion of the cellular prion protein (PrP^{c}) into an aggregated form (PrP^{Sc}) that self-propagates by imposing its abnormal conformation onto PrP^{c} molecules. Previous attempts to identify anti-prion compounds aimed to reduce the load of PrP^{Sc} aggregates by decreasing their stability or increasing their clearance. Some of these compounds showed potent activity *in vitro* or in cultured cells, but little or no efficacy *in vivo*. Multiple pieces of evidence support the notion that PrP^{c} loses its native fold in the initial steps of the aggregation process. This concept provides a rationale for tackling PrP^{c} aggregation by stabilizing the monomeric protein precursors, instead of disrupting pre-formed PrP^{Sc} species. The underlying idea is to block aggregation by increasing the Gibbs free energy barrier (δG) required for the initial misfolding events. This goal could be achieved with small, high affinity ligands of PrP^{c}, capable of acting as pharmacological chaperones. In order to identify such compounds, we developed a novel screening method based on Dynamic Mass Redistribution (DMR), a label-free, fully automated biophysical technique performed on 384-well microplates, and capable of detecting molecular interactions at the equilibrium. We first employed DMR to test the interaction of a small set of previously reported anti-prion compounds to PrP^{c}. Our analyses confirmed, refined or disputed previous data, defining accurate binding constants for each molecule. These results demonstrated that DMR is a reliable platform for the identification of novel PrP^{c} ligands. Results from an ongoing DMR-based, high throughput screening campaign aimed at identifying new high-affinity ligands for human recombinant PrP^{c} will be reported.

P-002: Using small molecule reagents to help distinguish among prion structural models

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The only demonstrated difference between infections prions (PrP^{Sc}) and the isosequential

normal cellular prion protein (PrP^{c}) is conformation. The structure of PrP^{Sc} has been determined by a variety of instrumental techniques. The structure of prions remains uncertain. Recent instrumental analysis has led researchers to conclude that the secondary structure of prions is composed almost entirely of -sheet. Although this new analysis clarified an element of the secondary structure, it did not define the nature of the -sheet arrangement.

In order to better understand their structure, prions were reacted with a small molecule reagent. Five strains of hamster-adapted scrapie (Sc237 (=263K), drowsy, 139H, 22AH, and 22CH) and recombinant PrP were reacted with 5 different concentrations (0, 1, 5, 10, or 20 mM) of the reagent (N-hydroxysuccinimide ester of acetic acid (Ac-NHS)). The extent of lysine acetylation by Ac-NHS was quantitated by mass spectrometry. We determined that each of the lysines in rPrP reacts similarly. However, each of the lysines in the strains reacts differently from the other lysines in a given strain. Furthermore, the lysines in the strains react in a strain-dependent manner. Lysines in the C-terminal region of prions have different strain-dependent reactivity.

The results are consistent with one of the 2 recently proposed models for the structure of a prion. One model proposes that prions are composed of a 4-rung ß-solenoid structure comprised of 4 ß-sheets that are joined by loops and turns of amino acids. In the ß-solenoid structure, amino acids can project toward the center of the solenoid or outward. This could account for the difference in their reactivity. An alternative structure proposes that prions are composed of parallel in-register intermolecular β-sheets (PIRIBS). In such a structure the amino acid side chains would all be surface exposed and would be expected to react in a similar fashion. Variation in the amino acid composition of the loops and ß-sheet structures in the ß-solenoid structure is thought to result in different strains of prions.

P-004: Structural characterization of ex vivo mammalian prions isolated from multiple strains

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Until now, the 3-dimensional structure of infectious mammalian prions and how this differs from non-infectious amyloid fibrils remained unknown. Mammalian prions are hypothesized to be fibrillar or amyloid forms of prion protein (PrP), but structures observed to date have not been definitively correlated with infectivity. One of the major challenges has been the production of highly homogeneous material of demonstrable high specific infectivity to allow direct correlation of particle structure with infectivity.

We have recently developed novel methods to obtain exceptionally pure preparations of prions from prion-infected murine brain and have shown that pathogenic PrP in these high-titer preparations is assembled into rod-like assemblies (Wenborn et al. 2015. Sci. Rep. 10062). Our preparations contain very high titres of infectious prions which faithfully transmit prion strain-specific phenotypes when inoculated into mice making them eminently suitable for detailed structural analysis. We are now undertaking structural characterization of prion assemblies and comparing these to the structure of non-infectious PrP fibrils generated from recombinant PrP.

P-005: Lack of stress protection by the cellular prion protein: An alternative role in regulating growth factor signaling

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A thorough understanding of cellular prion protein (PrP^c) function will help determine whether loss of this function plays a role in the pathogenesis of transmissible spongiform encephalopathies (TSE). Although a wide array of functions have been proposed, the common theme linking together much of the research to date is that PrP^c protects cells from stress. In contrast, we have found that stable transfection of the prion protein gene into SH-SY5Y neuroblastoma cells does not mediate robust protection against several chemical toxins that cause different forms of stress. Two out of 4 stably-transfected clones did show enhanced survival relative to untransfected cells that do not express detectable levels of PrP^c. However, knockdown of PrP^c using a small interfering RNA (siRNA) had no effect on the resistance to stress, indicating that it was not mediated by PrP^c. Moreover, when cells were deprived of serum, we found that 3 out of 4 clones displayed reduced viability relative to untransfected cells. Given the clear lack of stress protection afforded by PrP^c transfection, we carried out proteomic analyses of the cells to identify alternative processes regulated by PrP^c. These experiments highlighted roles in cytoskeletal organization and cell cycle regulation. Differential expression of several proteins involved in cytoskeletal organization, such as vimentin, caldesmon and zyxin, were validated by western blotting. Additionally, we found that the altered cell cycle regulation of the stably-transfected clones caused them to proliferate slower than untransfected cells, an effect that was partially rescued by siRNA-mediated knockdown of PrP^c expression. The proteomic changes in the transfected cells are consistent with differential regulation of specific growth factor-activated pathways that we are beginning to dissect. Importantly, a role for PrP^c in regulating such pathways could explain many of the proposed functions for the protein and may indicate that loss of PrP^c function is an important factor in TSE pathogenesis.

P-006: Structural and folding studies of the protective V127 variant of human prion protein

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The V127 variant of human prion protein (PrP), first identified in the Kuru-affected region of Papua New Guinea, in which the highly conserved glycine at residue 127 is substituted for valine, has recently been shown to provide total protection against prion disease (Asante et al Nature 2015). These transmission studies indicate that V127 PrP is intrinsically resistant to prion propagation and furthermore can inhibit propagation involving wild-type PrP. This mutation acts via a different mechanism to the other common protective PrP polymorphism at residue 129, where heterozygosity is protective. As a first step in characterizing the protective effect of this polymorphism, we determine the 3-dimensional structure of the cellular form of PrP carrying this V127 mutation on both residue 129 polymorphic backgrounds. In concert with this, we characterize the stability and unfolding characteristics of these PrP variants containing the protective V127 mutation.

P-007: Non-equivalent binding sites for Abeta1-40 on PrP determine the oligomerisation pathway

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Normal prion protein (PrP^C) has been proposed to play an important role in the features of Abeta1-42 assemblies and their cytotoxicity in Alzheimer disease (AD). Even if PrP^C may not affect either the amount of Abeta plaques or the astrogliosis associated with the evolution of AD in transgenic mice, it plays an important role in behavior impairment and cognitive deficits.

The involvement of PrP^C in AD requires a physical interaction between PrP^C and Abeta. The effect of this interaction has been extensively explored in the context of AD, but little is known about the effect of this interaction on PrP structural dynamics. In the present work, we explored the effect of the PrP: Abeta1-40 interaction on the PrP oligomerisation process. In contrast to the PrP: Abeta interaction, which reduces Abeta aggregation, we observed a global enhancement of PrP oligomerisation in the presence of monomeric Abeta1-40. Moreover, we demonstrated that the PrP sites involved in Abeta binding are non-equivalent, and although a global increase in PrP oligomerisation was observed, Abeta1-40 binding to the 2 extraglobular domain sites had an antagonistic effect. Finally, by identifying the structural pathway through which Abeta1-40 affects PrP oligomerisation, we demonstrated that Abeta1-40 acts as an allosteric effector. These results provide new insights into the mechanisms of the physiologically relevant PrP: Abeta1-40 interaction as well as the key structural changes that may be involved in PrP signal transduction.

P-008: Proteolytic shedding of PrP^c: Giving a little to gain a lot?

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The membrane-anchored cellular prion protein (PrP^c) plays detrimental roles in neurodegeneration by its ability to misfold into a pathogenic isoform (in prion diseases) and by acting as a neuronal receptor for toxic protein oligomers (e.g., in Alzheimer's disease). On the contrary, released forms of PrP^c may have protective functions. Mature PrP^c is processed by a variety of proteolytic cleavage events. Given the high conservation and broad occurrence of these events, a picture is arising in which the differential cleavage of PrP^c affects its physiological functions and influences its roles in neurodegenerative diseases. Accumulating data from different laboratories indeed support this model.

For one of these cleavages, the release of PrP^c from the neuronal surface by action of the metalloprotease ADAM10,[1] we have recently shown significant modulation of several pathophysiological aspects in prion disease mouse models.[2] Fitting to the scenario described above, ADAM10 positively influences survival[2,3] likely due to 2 main reasons: (i) reduction of PrP^c at the neuronal surface and (ii) generation of a protective soluble form of PrP^c. To facilitate reliable detection of the latter, we have generated a novel antibody that shows high specificity and sensitivity for shed PrP in cell culture supernatants and murine brain. By

using this antibody we have gained deeper insight into various mechanistic aspects of ADAM10-mediated shedding of PrP^c. Here, we will report our most recent findings on the physiological and pathological relevance of this processing step and present an update on our experimental strategy of exploiting this cleavage as a therapeutic option in prion diseases and other devastating proteinopathies as well.

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P-009: Calibration of ultrasonic power and conformational analysis of MoPrP amyloid fibrils

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Ultrasonication has been widely used to amplify the scrapie from of prion protein, or other amyloids *in vitro*. Firstly, to elucidate the effects of ultrasonication on the formation of amyloid fibrils, we determined the ultrasonic power using both calorimetry and KI oxidation. These methods revealed that the ultrasonic power in our system was ranged from 0.3 W to 2.7 W. Based on the proper calibration of the ultrasonic power, the amyloid formation of MoPrP was investigated. The nucleation time of amyloid fibrils was found to be shortened almost proportionally to the ultrasonic power, indicating that the probability of the occurrence of nucleus formation increases proportionally to the ultrasonic power. Although amyloid fibrils were formed early at the strong ultrasonic power larger than 2.6 W, fine fragmentation of amyloid fibrils occurred. Thus, a balance between the extension and the fragmentation of the preformed amyloid fibrils is essential.

Subsequently, to characterize the conformation of MoPrP fibrils formed under proper ultrasonic irradiation, we synthesized position-specific double-fluorescence labeled MoPrP for a FRET analysis. The result indicated that a distance between fluorescence labeled N- and C-terminal sites of MoPrP increased upon the formation of amyloid fibrils compared with that of the native state. These approaches proposed here, calibration of ultrasonic power using calorimetry/KI oxidation methods and conformational analysis using FRET spectroscopy, are useful for amplifying prions *in vitro* and elucidating the conformation of abnormal PrP, respectively.

P-011: Matrix metalloprotease processing of the prion protein

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Post-translational processing of the cellular prion protein (PrP^C) includes several

proteolytic cleavage events, namely the well-described α- and β-cleavages, cell surface PrP^C shedding, and the newly described gamma-cleavage. The precise purpose of PrP^C endoproteolysis is not yet understood, though there is increasing evidence for distinct biological roles of the full-length protein and various N- and C-terminal fragments, as well as links to prion disease susceptibility, pathogenesis and misfolded prion protein (PrP^{Sc}) propagation. The dominant PrP^C proteolytic cleavage event in normal (healthy) cells is α-cleavage, which occurs within the potentially neurotoxic and amyloidogenic central region of PrP^C, producing the N1 and C1 fragments. Despite this, the exact identity of the protease responsible for α-cleavage is contentious, with evidence both for and against the involvement of the ADAMs family of proteases. We previously reported the identification of the matrix metalloprotease (MMP) family of proteases as being key regulators of PrP^C endoproteolysis (Prion2015), in particular MMP2, which was capable of producing C1 from full-length recombinant human PrP (recPrP) *in vitro*. Additionally, treating cultured cells with prinomastat, a pan-MMP inhibitor, somewhat counter-intuitively significantly increased C1 levels, and decreased PrP^{Sc} levels in M1000 prion infected cells.

These novel and dichotomous findings prompted our continued investigation of the role of MMPs in PrP^C processing and prion propagation, also incorporating the comparison of biologically relevant PrP^C variants (codon 129M/V and codon 127G/V). Utilizing the recombinant protein *in vitro* assay we found that similar to MMP2, MMP7, and to a much lesser extent MMP9, also cleave PrP, largely at the α-cleavage site. We observed several differences in the processing of recPrP by the various MMPs, including differences in the proteolysis of the recPrP N-terminus, the digestion of higher molecular weight (dimerized) recPrP species, and the involvement in recPrP gamma-cleavage. Interestingly, we also detected differences in the processing of cell derived PrP^C and PrP^{Sc} by the recombinant MMPs. Of possible relevance to prion diseases, the 2 codon 129 variants showed similar MMP cleavage profiles, however in contrast, the codon 127V recPrP appeared less susceptible to MMP-induced gamma-cleavage. Deduction of the mechanistic pathway/s of MMP-regulated prion protein proteolysis, and its impact on normal PrP^C function and prion diseases, are the subject of ongoing investigation.

P-012: A ZIP6-ZIP10 heteromer interacts with NCAM1, controlling its phosphorylation and integration into focal adhesion complexes during epithelial-to-mesenchymal transition

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The prion protein evolved from the sub-branch of ZIP metal ion transporters comprising ZIPs 5, 6 and 10, raising the spectre that the study of these zinc transporters may reveal insights relevant for understanding the function of PrP. This concept is supported by (i) data documenting an interaction of PrP with ZIP6 and ZIP10 and by (ii) observations of morpholino-based knockdown of PrP or ZIP6 disrupting a cellular program known as epithelial-to-mesenchymal transition (EMT) during zebrafish gastrulation. We recently documented that PrP is critical for the execution of EMT also in mammalian cells and controls a signaling loop that culminates in the polysialylation of the neural cell adhesion molecule 1 (NCAM1). Here, we investigated ZIP6 in the same cell model using CRISPR-Cas9-mediated ZIP6 knockout clones, mass spectrometry and bioinformatic methods. Reminiscent of PrP, ZIP6

levels are 5-fold upregulated during EMT and the protein forms a complex with NCAM1. ZIP6 also interacts with ZIP10 and these 2 ZIP transporters co-regulate each other's expression, adding weight to the notion that PrP's ability to interact with these ZIP proteins reflects a propensity of ZIP/PrP family members to form heteromeric complexes. Interestingly, ZIP6 mediates phosphorylation of NCAM1 on a cluster of cytosolic phosphoacceptor sites and is critical for integration of NCAM1 in focal adhesion complexes but, unlike cells lacking PrP, ZIP6 deficiency does not abolish polysialylation of NCAM1. Bioinformatic analyses suggest that the interaction between NCAM and ZIP proteins with PrP-like ectodomains preceded the evolutionary invention of prion genes and polysialyltransferases mediating NCAM1 polysialylation by a half billion years. Our data are consistent with a model whereby PrP inherited its ability to interact with NCAM1 from its ZIP ancestors but acquired a specialized role in NCAM1 polysialylation as an adaptation to the emergence of polysialyltransferases in the vertebrate lineage.

P-013: Dimer-sized PrP^{Sc} formation detected by western blotting

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During the screening of anti-prion compounds in persistently prion-infected cells, we found treatment of the cells with certain compounds produced new bands of dimer-sized PrP^{Sc} which likely contained covalently bonded PrP molecules in protein gel blot analysis. The compounds include thiazoline derivatives and Congo-red, which enhanced the dimer-sized PrP^{Sc} formation in either prion-infected cells or cell lysates in a time and does dependent manner. Urea, SDS and reducing compounds such as 2ME did not disrupt the dimer-sized PrP^{Sc} signals. All anti-mouse PrP antibodies tested in the study recognized the dimer-sized bands as well as monomer bands. Treatment with the compounds did not produce dimer-sized PrP molecules from the cell lysates containing only PrPc or PK-digested PrP^{Sc}. Excess exposure of PrP^{Sc}-containing cell lysates to the compounds, however, reduced the whole amount of PK-resistant PrP^{Sc} in western blot analysis. Because the dimer-sized PrP^{Sc} formation by the compounds occurred in crude cell lysates and was not inhibited by the presence of competitive amino acids, it is suggested that the dimer-sized PrP^{Sc} formation occurs in a fashion unlike to conventional cross linker chemistry. If the dimer-sized PrP^{Sc} is composed of PrP molecules alone, the findings imply that covalently stable dimer formation of PrP^{Sc} molecules may occur because of their closely contacted aggregation nature in either live cells or cell lysates.

Finally, it remains to solve the following questions: what is the chemical structure of dimer-sized PrP^{Sc}, and which sites of PrP are involved in the dimer-sized PrP^{Sc} formation? Answers to these questions might give a clue to elucidating the mechanism of prion propagation at a molecular level.

P-014: The Prion Protein and genotoxic stress

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The physiological roles of the cellular prion protein (PrP^{C}) are incompletely understood, despite comprehensive investigations. However, lines of evidence suggest that the protein contributes to cellular survival under stressful conditions, such as serum deprivation[1] and exposure to reactive oxygen species (ROS).[2] More recently, PrP^{C} has been attributed a critical role in DNA repair and protection of cells against genotoxic stress

through direct interaction with the endonuclease APE.[1,3] To explore this further, we have utilized white blood cells derived from goats with and without expression of PrP^{C}[4,5] and human SH-SY5Y neuroblastoma cells. Since DNA repair mechanisms and cellular stress responses vary with different types of genotoxic stress, we have subjected cells to 3 modes of genotoxic stress. These stressors are: MMS inducing methyl adducts, doxorubicin inducing DNA double strand breaks and H2O2 generating oxidative DNA damage. We present data on cell viability and levels of DNA damage under these experimental conditions and discuss the influence of PrP^{C} on cellular responses to genotoxic stress.

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P-015: Effects of cell growth suppression treatments on PrP^{Sc} accumulation in prion-infected cells; paradoxical phenomena observed in butyric acid treatment

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Prion diseases are fatal neurogenerative disorders caused by PrP^{Sc} accumulation in the brain. The mechanism of PrP^{Sc} accumulation in neuronal cells is yet to be fully clarified and is an important issue to be solved for better understanding the prion.

In this study, we aimed to getting a clue to this mechanism by using cell growth suppressed, prion-infected cells. We used both 22L prion-infected neuro2a cells and the cells from which PrP^{Sc} had been removed by pentosan polysulphate (PPS) treatment. Cell growth of these cells was suppressed in a medium of low serum and low glucose, by the treatment with retinoic acid, butyric acid (BA), or hexanoic acid. From the comparison between the PPS-untreated and PPS-treated cells, we found that BA treatment increased the level of PrP^{Sc} but paradoxically decreased the level of PrP^{C} in the cells. Because BA treatment of the cells decreased the level of autophagosome-specific LC3-II, it is suggested that BA treatment suppresses the degradation process of PrP^{Sc}. Regarding the paradoxical change in PrP^{C} level, we are examining the turnover and localization of PrP^{C} to understand what happened to PrP^{C} metabolism in the cells treated with BA.

BA is known as a histone deacetylase inhibitor, and BA treatment is reported to cause regeneration of dendrites and synapses and to recover learning ability and long-term memory in neurodegenerative mice. In this study, BA treatment of the cells consistently increased the expression levels of MAP2, a cytoskeletal protein enriched in dendrites, and Synapsin I, a synaptic vesicle associated protein. On the

other hand, we found that BA treatment decreased the expression level of β-actin of the cells. Therefore, we are also currently examining potential relationships between this β-actin suppression and described phenomena caused by BA treatment of the cells.

In our paper presentation, we will show further data and discuss the mechanism of paradoxical phenomena already described.

P-017: Semisynthesis of lipidated prion protein variants

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The prion protein (PrP) is a pathogen involved in infectious, neurodegenerative disorders, so-called prion diseases or transmissible spongiform encephalopathies (TSEs).

Prion diseases are based on the conversion of cellular (PrPc) into misfolded, aggregated scrapie prion protein (PrP^{Sc}) on the outer leaflet of the cell membrane.[1,2]

Membrane attachment via C-terminal glycosylphosphatidylinositol (GPI) anchor seems to be crucial for prion toxicity.[3]

In order to investigate the mechanisms of PrP conversion and toxicity, conformational changes, localization and orientation of PrP on the membrane and during cellular processing shall be determined by using semisynthetic, fluorescently labeled PrP variants. These PrP variants will be analyzed using fluorescence microscopy, infrared spectroscopy and surface plasmon resonance (SPR) on lipid layers.

Here we present results of a semisynthetic approach that is based on expressed protein ligation (EPL) to produce recombinant full length (FL, aa23-231), N-terminally truncated (T, aa90-231), central region deleted (δCR, aa23-104/126-231) PrP thioesters linked to GPI-mimicking labeled and non-labeled peptides.[4-8]

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P-018: In vitro seeding of amyloid plaques

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The mechanism of amyloid plaque formation in protein misfolding neurodegenerative diseases, including Alzheimer disease and Prion diseases remains largely unknown. It is currently believed that pre-formed aggregates of misfolded protein may seed the formation of fibrils which then aggregate to form amyloid plaques.

Whole brain organotypic slice culture (BOSC) has been utilised here to enable real-

time modeling of plaque formation dynamics in Alzheimer disease and prion disease models *in vitro*. Using a combination of LI-COR and confocal imaging systems whole BOSC have been characterized and incubated with prion infected brain homogenate, recombinant prion protein fibrils and β amyloid seeds.

Brain slices have been maintained in culture for up to 8 months, during which time we have imaged changes in specific cell populations such as neurons and glia using fluorescent immunohistochemistry in combination with confocal microscopy. Viable neurons were seen at 8 months and there is a rapid and consistent appearance of a glial cap or scar which encases the exposed surface of the slice as a protective barrier to the environment. A comprehensive morphological analysis of slices have been performed to allow changes in cell morphology in response to the application of pre-formed seeds to be determined. Cell stress and survival within BOSC have been evaluated following Prion infection and stress levels appear to be elevated within infected BOSC compared to un-infected controls. We have successfully demonstrated replication of infectivity within whole BOSC following prion infection after 2 months in culture.

To compare plaque formation *in vitro* and *in vivo*, APP transgenic mice (J20) have been inoculated with brain homogenate, recombinant prion protein fibrils and β amyloid seeds to evaluate *in vivo* seeding potential and compare with that observed in BOSC.

This study has produced a well characterized *in vitro* model which has multiple applications, including the investigation of amyloid plaque seeding, due to its accessibility and ease of manipulation. Use of whole BOSC for preliminary experimental investigations has the potential to generate valuable data which can direct future *in vivo* studies. By gaining insight into biological systems initially at the *in vitro* level the BOSC model may help to increase our basic knowledge of the brain with less of the complexity that appears with *in vivo* models.

P-019: Discovery of anti-prion agents using a PyMOL plugin-based logical drug design platform NAGARA

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Logical drug design is a strategy for designing small compounds called medical chaperones (MCs) that stabilize the conformation of a target protein. To accelerate the design procedure, we developed a plugin called "NAGARA" based on the PyMOL program, and applied it to the discovery of MCs that stabilize the cellular form of a prion protein (PrP^c). In NAGARA, we constructed a single platform to unify the docking simulation (DS), free energy calculation by molecular dynamics (MD) simulation, and interfragment interaction energy (IFIE) calculation by quantum chemistry (QC) calculation. NAGARA also enables large-scale parallel computing via a convenient graphical user interface. Here, we demonstrated its performance and its broad applicability from drug discovery to lead optimization with full compatibility with various experimental methods including Western blotting (WB) analysis, surface plasmon resonance (SPR), and nuclear magnetic resonance (NMR) measurements. Combining DS and WB, we discovered anti-prion activities for 2 compounds and tegobuvir (TGV), a non-nucleoside non-structural protein NS5B polymerase inhibitor showing activity against hepatitis C virus genotype 1. Binding profiles predicted by MD and QC are consistent with those obtained by SPR and NMR. Free energy analyses showed that these compounds stabilize the PrP^c conformation by decreasing the conformational fluctuation of the PrP^c. Because TGV has been already approved as a medicine, its extension to prion diseases is straightforward. Finally, we evaluated the affinities of the fragmented regions of TGV using

QC and found a clue for its further optimization. By repeating WB, MD, and QC recursively, we were able to obtain the optimum lead structure.

P-020: *Zinnia elegans* combined PrP^{BSE} complex increases the survival time of VM mice

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Bovine spongiform encephalopathy (BSE) prions are infectious neurodegenerative disorder in cattle, which are characterized by the accumulation of an abnormal form of prion protein (PrP^{BSE}) that is partially resistant to degradation by protease. There are currently no proven therapeutic agents for BSE although several researchers have been searching for anti-prion drugs. We previously found the new antiprion natural compounds (*Rubus coreanus Miquel*, *Zinnia elegans*) that can inhibit PrP^{BSE} in a persistently PrP^{BSE} infected cell line M2B. Our study investigated the ability of *Zinnia elegans* ethanol (ZEE) extract to degrade PrP^{BSE} *in vitro* from BSE-infected brain. The samples combined ZEE extracts with prion protein were designated as ZEE binding normal brain homogenate and ZEE binding PrP^{BSE} brain homogenate, ZEE-BH and ZEE-PrP^{BSE} BH, respectively. We also observed that survival periods of the mice were inoculated intracerebrally with ZEE-BH or ZEE-PrP^{BSE} BH. We compared dose-dependence samples of PrP^{BSE} that had been reacted with ZEE extracts for 1 hour. ZEE-PrP^{BSE} BH had substantially reduced PrP specific bands and not been amplified PrP^{BSE} by sPMCA with 8 mg/mL *in vitro*. Survival period of each group was 130±3 days (vehicle), 167±21 days (ZEE 4 mg/mL) and 174±3 days (ZEE 8 mg/mL). We identified significant increase (P value: < 0.0001) between survival period of control group and each ZEE-PrP^{BSE}BH group (compared each survival period by log rank test). In fact, PrP^{BSE} level of brain from all experimental groups at terminal stage was represented the similar pattern by immunodetection. It was shown that the survival time in animals treated ZEE-PrP^{BSE} BH was prolonged and the ZEE extracts were shown degradation of PrP^{BSE}. These results indicated that treatment of ZEE-PrP^{BSE} BH extended for survival period in VM mice. Further animal experiment is necessary to confirm the effect of this BSE inhibitor in clinical studies.

P-021: Gene expression profiling analysis of *Rubus coreanus Miquel*-cured prion-infected MDBK cell line

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Bovine spongiform encephalopathy (BSE) is a neurodegenerative disorder in cattle and a member of the transmissible spongiform encephalopathies (TSEs), also known as prion diseases. TSEs are characterized by the accumulation of an abnormal form of prion protein (PrP) that is partially resistant to degradation by protease and invariably fatal. We identified the natural compound, *Rubus coreanus Miquel* (RCM) that can inhibit PrP^{BSE} in MDBK cells persistently infected with BSE (termed M2B) from the previous experiment. In this study, we performed microarray analysis and examined the differentially expressed (DE) genes between BSE-infected and RCM-cured M2B cell line for anti-inflammatory function. Microarray analysis identified 26 genes that were found to be differentially expressed. We also observed differential expression in several genes in the inflammatory cytokines and

receptor pathway. Thirty nine percent (33/84) of genes showed >2 fold differential expression in each of the 3 examined samples. The expression profile of some genes identified in the inflammation array by our pathway-focused analyses was classified into 6 main groups, referred to as Chemokine (C-C motif) ligand (CCL), Chemokine (C-C motif) receptor (CCR), Chemokine (C-X-C motif) ligand and receptor (CXCL&CXCR), Type I cytokine family, Type II cytokine family and TNF family & MIF-3. The selected genes including CCL5, CCL20, IL8, IL17 and CXCR2 from microarray assay experiments showed significant changes in expression at a high frequency in agreement with the results of the real-time PCR-Base array analysis. These identified genes from pathway-focused gene expression profiling assay would be used to understand the molecular study for prion infection related to inflammation.

P-022: The effects of PrP^C glycosylation and cofactor molecules on species-specific prion strain susceptibility in the bank vole

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Bank voles are uniquely susceptible to a number of prion strains and appear to lack a traditional species barrier. Transgenic mice expressing bank vole PrPc retain these unusual characteristics, indicating that susceptibility is encoded within the bank vole's PrP primary amino acid sequence.[3] However, the mechanistic basis for the universal susceptibility of bank vole PrP^C remains unknown.

We are using a biochemical approach to study the mechanism used by bank vole PrP^C to convert into PrP^{Sc} *in vitro*. Our lab has previously shown that species that have *in vivo* transmission barriers, such as mouse and hamster, possess unique and specific cofactor and glycosylation requirements for propagation of prions *in vitro*.[1,2] Therefore, we hypothesize that bank vole PrPc may have less restrictive glycosylation and cofactor requirements. Bank vole PrP may be versatile with regard to PrP^C glycosylation status and cofactor specificity. Alternatively, cofactor usage and glycosylation requirements may be seed-dependent.

To study these questions, we will use reconstituted PMCA to compare the effect of prion seeds from different species on cofactor and glycosylation requirements for bank vole PrP conversion *in vitro*. This study presents a unique opportunity to test constraints on propagation in a universal acceptor of prions.

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P-023: Autophagy is needed in opposing roles in the life cycle of prions and also impacts exosomal release of prions

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Autophagy is a basic cellular program for degradation and recycling of cellular components which can exert both beneficial and adverse effects in various neurodegenerative diseases. We tested the role of autophagy in prion-infected cells and how this affects the life cycle of prions. In non-neuronal cells we found a pronounced dependence of prion replication on autophagy competence, suggesting that autophagy provides a disaggregase function as needed in prion conversion and propagation. When we infected mouse embryonic fibroblasts (MEF) with various strains of prions we observed a very pronounced up-regulation of autophagy markers when cells started propagating prions at detectable levels in immunoblot analysis. MEFs deficient in autophagy (ATG5-/-) were severely hampered in prion propagation, which could be rescued by re-introduction of Atg5. Surprisingly, in prion-infected neuronal cells (ScN2a and ScCAD5) autophagy had an opposite role. In cells compromised in (via Atg5 or beclin-1 si/shRNA knockdown) or ablated for autophagy competence by CRISPR/Cas9-mediated gene editing (ATG5-/-), autophagy competence was dispensable for prion infection. Obviously, in such cells autophagy was used for prion degradation and compromised autophagy resulted in even elevated levels of PrP^{Sc}. This data shows that autophagy is used for at least 2 functions in the life cycle of prions, and depending on the cellular context this can be propagation or degradation of prions. Given the central role of late endosomes in both autophagy and exosomal release machinery we then tested whether autophagy controls also exosomal release of PrP^{Sc}. Characterizing exosomal preparations from prion-infected cells impaired or stimulated in autophagy very clearly showed that induction of autophagy reduces exosomes and exosomal PrP^{Sc}, whereas a compromised autophagy does the opposite. Taken together, these findings validate induction of autophagy in neuronal cells as a promising therapeutic strategy for prion and prion-like diseases.

P-024: Polymorphism of PrP amyloid-like fibrils can be defined by the concentration of seeds

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Prions are infectious proteins where the same protein may express distinct strains. The strains are enciphered by different misfolded conformations. Strain-like phenomena have also been reported in a number of other amyloid-forming proteins. One of the features of amyloid strains is the ability to self-propagate, maintaining a constant set of physical properties despite being propagated under conditions different fromthose that allowed initial formation of the strain. Here we report a cross-seeding experiment using strains formed under different conditions. Using high concentrations of seeds results in rapid elongation and new fibrils preserve the properties of the seeding fibrils. At low seed concentrations, secondary nucleation plays the major role and new fibrils gain properties predicted by the environment rather than the structure of the seeds. Our findings could explain conformational switching between amyloid strains observed in a wide variety of *in vivo* and *in vitro* experiments.

P-025: Restricted propagation of sheep scrapie in hamsters

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Prion diseases are a group of neurodegenerative diseases that are inevitably fatal. Prions are comprised of a misfolded protein (PrP^{Sc}) derived from the host protein, PrP^{c}. Golden Syrian hamsters are susceptible to infection with prions from a wide range of species including, mink, rodents, cervids, cattle and humans. Hamsters are susceptible to sheep scrapie if first passaged through mice and rats. Direct transmission of scrapie to hamsters has not been shown. In this study we investigated the susceptibility of hamsters to sheep scrapie infection by both *in vivo* and *in vitro* methods. Groups of hamsters were intracerebrally inoculated with sheep brain from either a scrapie infected or uninfected animal. The hamsters failed to develop clinical signs of prion disease at 690 d post infection at which time the experiment was terminated. Consistent with the clinical diagnosis, Western blot failed to detect PrP^{Sc} in brain tissue from these animals. To further explore the susceptibility of hamsters to sheep scrapie infection, *in vitro* experiments were performed using protein misfolding cyclic amplification (PMCA). Using hamster brain homogenate as the substrate, we seeded the PMCA reactions with sheep brain homogenate from either scrapie infected or uninfected sheep. In the mock seeded PMCA reactions, we failed to detect PrP^{Sc} through 12 rounds of PMCA. In the scrapie seeded PMCA reactions, we failed to detect PrP^{Sc} by the 12 round of PMCA in 5 of the replicates, while one replicate became PrP^{Sc} positive at round 8 of PMCA. In conclusion, our results suggest that a large species barrier between sheep and hamster exists.

P-026: Comparison of the *in vitro* seeding activity of UK iatrogenic and sporadic Creutzfeldt-Jakob disease subtypes by real time quaking induced conversion

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Human prion diseases are a group of neurodegenerative conditions associated with misfolding and aggregation of a host protein (PrP^{c}) to a pathogenic form (PrP^{Sc}). The clinico-pathological features of the most common human prion disease, sporadic Creutzfeldt-Jakob disease (sCJD) are closely associated with the host *PRNP* codon 129 genotype (MM, MV or VV) and the PrP^{Sc} type as determined by Western blotting of protease resistant prion protein (PrP^{res} type 1 or 2). Iatrogenic CJD (iCJD) is an acquired form of the disease that results from the transmission of prion infection from person-to-person during medical or surgical treatment. Cases of iCJD have been associated with treatment using human growth hormone derived from cadaveric pituitaries (hGH-iCJD), and also the use of dura mater grafts during neurosurgery (DM-iCJD). It is presumed that the hormone or grafts were contaminated with tissue from individuals who died from sCJD. It has been hypothesized that secondary transmission of a human prion diseases could result in acquired virulence. In this study we have compared a molecular property of the pathogenic agent between sCJD and hGH-iCJD, namely its ability to seed conversion of normal PrP to a misfolded form. To do this we have used the lag time to conversion in the real-time quaking induced conversion (RT-QuIC) assay as a measure of prion seeding activity. Cerebral cortex tissue samples from 20 hGH-iCJD cases were analyzed alongside samples from a comparable number sCJD patients. The cases compared were matched for subtype as defined by *PRNP* codon 129 genotype and PrP^{res} type. The hGH-iCJD cases as a whole and their molecular subtypes (VV2, MV2 and MM1) had a

lower mean seeding activity compared with the sCJD cases as a whole and their corresponding molecular subtypes. Cases of DM-iCJD also had a lower seeding activity compared with sCJD cases of the same subtype (MM1). These results suggest that human-to-human transmission of prion disease may affect the seeding properties of PrP^{Sc} associated with the disease, even when the PrP^{res} isotype appears to be maintained. The results are not consistent with the hypothesis that secondary transmission of a human prion disease results in acquired virulence, at least as judged by seeding activity in the RT-QuIC assay.

P-027: PrP glycosylation-independent amplification of prions using highly efficient cell-based protein misfolded cyclic amplification

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Prions are pathogens composed of misfolded assemblies (PrP^{Sc}) of the variably N-glycosylated, cellular prion protein (PrP^{C}). Within the brain of infected species, prions replicate by seeding the conversion and polymerization of host PrP^{C}. Distinct prion strains exhibit specific PrP^{Sc} biochemical properties and specific biological traits in defined host-species. Strain information is encoded within the conformation of PrP^{Sc} assemblies. The molecular requirement for PrP^{Sc} formation in specific tissue and maintain of strain information through passaging, including notably the defined glycotype of PrP^{Sc}, remain mostly unknown. Here, by cell modeling and *in vitro* conversion, we examined whether glycans are involved in the perpetuation of strain information and the reason for defined stoichiometric ratio of PrP^{Sc} glycoforms.

First, we replaced brain by cell lysate from cells expressing different PrP^{C} species (including human) as substrate to yield high rate of prion conversion in a test tube by protein misfolding cyclic amplification (PMCA). The total protein concentration appeared instrumental to achieve the highest the amplification efficacy, allowing reduction and even bypassing the use of brain substrate. In ethical and practical terms, sensitive PMCA can thus be performed without requiring animal models.

Second, we submitted 127S scrapie prion seeds to PMCA with cell lysates expressing solely mono- and/or un-glycosylated PrP^{C} mutants. This revealed that neither PrP^{C} nor PrP^{Sc} glycoform stoichiometry was instrumental to PrP^{Sc} formation and perpetuation of prion biological strain properties.

Our study supports the view that strain specified properties, including PrP^{Sc} glycotype are enciphered within PrP^{Sc} structural backbone, not in the attached glycans.

P-028: Validating human stem cell derived neural cultures as a flexible model system in which to investigate neurodegenerative mechanisms

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The fundamental mechanisms involved in chronic neurodegenerative diseases can be investigated using human tissue based

approaches, animal models and *in vitro* cellular and cell-free models. In practice, a combination of these approaches is usually required to study human disease mechanisms. In human prion disease research, a significant impeding factor has been the absence of a human cell culture model in which human prions replicate efficiently and reproducibly.

To address this issue, we have tested whether human induced pluripotent stem cells (iPSC) differentiated into mature astrocytes or neurons support prion replication. The iPSCs were derived from donors with no family history of neurological disorders and with no known disease-associated mutations, and were of known PRNP codon 129 genotype, including the 129MM genotype associated with variant Creutzfeldt-Jakob disease (vCJD). Cultures were exposed to a range of concentrations of vCJD brain-spiked media.

We found that PRNP codon 129MM astrocytes supported vCJD prion replication, whereas astrocytes of the PRNP codon 129MV genotype did not. The genotype-specificity of vCJD prion replication was validated in 2 ways: first, prion replication was observed in an independent 129MM codon iPSC line exposed to vCJD brain-spiked media. Second, we demonstrated that prion replication in PRNP codon 129MM cell lines was concentration-dependent. By contrast, PRNP codon 129MV cells were not competent to support vCJD prion replication, even at extremely high concentrations of vCJD prion-spiked media. We demonstrated $>90\%$ astrocytic viability immediately following exposure to vCJD brain spiked media. However, we also show some evidence that prion replication in PRNP codon 129MM astrocytes affects well-characterized astrocytic functions and molecular pathways. Finally, we have extended our findings to iPSC-derived cortical neuronal cultures.

Our study therefore addresses a long-standing gap in the repertoire of human prion disease research, and provides a model that can be used to investigate human prion disease mechanisms. In particular, specific functional changes arising in replication-competent astrocytes and neurons may provide insights into the role of the misfolded prion protein in neurotoxicity.

P-029: sCJD prion seeding activity in human urine by RT-QuIC

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Recently, a Protein Misfolding Cyclic Amplification (PMCA) protocol that is able to detect prions in urine from patients with neuropathologically confirmed variant Creutzfeldt-Jakob disease (vCJD) was published. The estimated sensitivity and specificity of this method were reported to be 93% and 100% respectively. However, using the same procedure, the authors were unable to amplify prions from the urine of patients affected by sporadic CJD (sCJD) and genetic forms of prion disease. PMCA is known to be very efficient in amplifying vCJD prions while it amplifies sCJD prions comparatively poorly. Conversely, the Real Time Quaking Induced Conversion (RT-QuIC) reaction is seeded very efficiently by sCJD brain homogenate and CSF samples whereas vCJD samples seed the reaction inefficiently. Starting from the RT-QuIC diagnostic protocol routinely performed in our laboratory on human cerebrospinal fluid samples, here we present preliminary data regarding RT-QuIC reaction optimization for the detection of seeding activity in urine from sCJD patients. We have conducted RT-QuIC analyses on neurological (non-CJD) patient urine spiked with sCJD brain homogenate. We assessed the effect of dialysis on the seeding efficiency and the detection limit of sCJD brain samples in our model of infected human urine. An RT-QuIC assay optimised for urine will allow us to ask whether the apparent absence of seeding activity in urine from sCJD patients is due to differences of pathophysiology between sCJD and vCJD, or whether it is due to the differing analytical sensitivities of RT-QuIC and PMCA for sCJD and vCJD samples.

P-030: Comparison of the *in vitro* amplification efficiency of UK iatrogenic and sporadic Creutzfeldt-Jakob disease subtypes by protein misfolding cyclic amplification

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Human prion diseases are a group of neurodegenerative conditions associated with misfolding and aggregation of a host protein (PrP^{c}) to a pathogenic form (PrP^{Sc}). The clinico-pathological features of the most common human prion disease, sporadic Creutzfeldt-Jakob disease (sCJD) are closely associated with the host *PRNP* codon 129 genotype (MM, MV or VV) and the PrP^{Sc} type as determined by Western blotting of protease resistant prion protein (PrP^{res} type 1 or 2). Iatrogenic CJD (iCJD) is an acquired form of the disease that results from the transmission of prion infection from person-to-person during medical or surgical treatment. Cases of iCJD have been associated with treatment using human growth hormone derived from cadaveric pituitaries (hGH-iCJD), and also the use of dura mater grafts (DM-iCJD). It is presumed that the hormone or grafts were contaminated with tissue from individuals who died from sCJD. UK cases of hGH-iCJD have occurred predominantly in the codon 129 MV and VV genotypes and are associated with type 2 PrP^{res}. It has recently been proposed that the phenotypic properties of the agent are partially retained when a human prion is transmitted across a codon-129 genotype barrier and that the origin of the infection may be inferred using an experimental *in vivo* "trace-back" phenomenon. In this study we have compared the ability of sCJD and hGH-iCJD cerebral cortex samples to convert PrP^{c} by protein misfolding cyclic amplification (PMCA), using humanised transgenic mouse brain substrates and Western blotting to detect PrP^{res}. PrP^{Sc} from sCJD and DM-iCJD of the MM1 subtype amplified poorly in PMCA, whether the substrate was codon 129MM or 129VV. In contrast, PrP^{Sc} from sCJD and hGH-iCJD of the VV2 subtype amplified more efficiently in PMCA, especially in the codon 129VV substrate. However, 2 rare cases of hGH-iCJD that were MM at codon 129 were available to test: one of these had a type 1 PrP^{res} and behaved in PMCA similarly to the sCJD and DM-iCJD MM1 subtypes. The other hGH-iCJD MM case had PrP^{res} type intermediate in mobility between type 1 and type 2 PrP^{res}. It amplified more efficiently in PMCA and had a preference for the codon 129VV substrate. These results are consistent with an *in vitro* "trace-back" phenomenon, evident in one out of the 2 hGH-iCJD codon 129MM cases available for study, and further suggests that the "trace-back" phenomenon may be mediated by a novel intermediate PrP^{Sc} conformational type.

P-034: Influence of a polymorphism in the highly conserved hydrophobic core region on chronic wasting disease prion propagation and pathogenesispathogenesis

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Chronic wasting disease (CWD) is the most contagious prion disease with substantial lateral transmission. The appearance of CWD in wild-living and migrating cervids makes it uncontrollable. Therefore, potential risk for human and other animals remains. Expression of PrP^{C} in the host is necessary for disease

development. Although the PrP primary structure is highly conserved between cervids, the disease phenotype can be modulated by species-specific polymorphisms in the prion protein gene.

When we compared the conformational stability of 2 CWD prion isolates from white-tailed-deer (WTD) using guanidinium denaturation, they exhibited a significant difference in the [GdnHCl] 50. Prnp genotyping revealed a polymorphism at codon 116 (A>G) in one animal, whereas the second animal harbored the wild-type (wt) PrP genotype. We used real-time quaking-induced conversion-assay (RT-QuIC) to compare the ability of wt- and 116AG-WTD prions to convert 4 recPrP substrates. We found that 116AG-WTD had a 100fold lower dilution endpoint with all substrates, although wt- and 116AG-WTD brain-homogenates showed comparable amounts of PrP^{Sc} in immunoblot. Moreover, using 116AG-WTD prions as a seed resulted in an extended lag (and log) phase and a lower maximum fluorescence level compared to wt-WTD. Propagation of PrP^{Sc} in primary neuronal cultures derived from transgenic mice overexpressing cervid PrP revealed a delay in conversion when 116AG-WTD brain-homogenate was used for infection compared to wt-WTD brain-homogenate. Interestingly, incubation time of disease was significantly prolonged using the same transgenic mice infected with 116AG-WTD, with a highly prolonged clinical phase. Although the [GdnHCl] 50 of PrP^{Sc} of transgenic mice inoculated with either of the 2 WTD isolates was increased, the difference in conformational stability between mouse-passaged wt- or 116AG-WTD prions was still significant and comparable to the original isolates. Similarly, the propagation of the mWTD in primary neuron cultures was comparable to the original isolates. Interestingly, results of molecular dynamics simulations revealed a less stable structure with additional β-strands and disruption of the native salt bridge network in the A116G-WTD-PrP.

The polymorphism at position 116 introduces a variation into the most highly conserved hydrophobic core region (HC) of PrP. The HC facilitates PrP^{C}-PrP^{Sc} interaction and the formation of β-sheet structure, and can act as a hinge region involved in prion conversion. Conformation is an important criterion when assessing transmission barrier, and conformational variants can target a different host range. Therefore, a thorough analysis of CWD isolates and re-assessment of species-barriers is important in order to fully exclude a zoonotic potential of CWD.

P-035: From misfolding to aggregation: Sequence effects on conformational properties of amyloidogenic peptides implicated in neurodegeneration

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There is an emerging evidence that many neurodegenerative diseases are characterized by the prion-like mechanisms of pathology at the cellular level. This includes cell-to-cell propagation of pathological conversion of amyloidogenic proteins, their possible sporadic misfolding due to different environmental factors (such as level of pH, intra-cellular crowding, interactions with lipids, among others). Inhibition of prion-like propagation of misfolding is an important strategy for developing the therapy against neurodegeneration.

The structural information on possible targets for therapeutic intervention in neurodegeneration remains illusive. It is currently accepted that small oligomers made of amyloidogenic proteins / peptides which are intermediates or off path products of formation of amyloid fibrils are neurotoxic agents in many neurodegenerative disorders, including the prion

diseases and Alzheimer disease. The information on molecular structure of neurotoxic oligomers can be used for optimization of specificity and affinity of conformational antibodies. Also, possible drugable sites of amyloidogenic proteins and their aggregates can be targeted to inhibit prion-like propagation of the pathological conversion.

Transient nature of neurotoxic oligomers significantly impedes their experimental characterization. Due to the large time and length scales involved, this a computationally challenging problem as well. To circumvent these difficulties, we developed a new multiscale platform for studying misfolding and aggregation pathways of amyloidogenic peptides based on the 3 dimensional molecular theory of solvation (aka 3D-RISM-KH). The approach provides efficient sampling of conformational space of proteins, and post-processing to account for the factors affecting misfolding and aggregation pathways (such as solvent conditions and intra-cellular crowding). Here, we use this new platform to study misfolding and aggregation of amyloid (A) β peptides implicated in Alzheimer disease, as well as the molecular structure of small Aβ oligomers.

We found significant differences in conformational states of Aβ (1-40) and (1-42) peptides. In particular, Aβ (1-42) peptides form more compact structures which can be explained in part by the difference in propensity to form intra-peptide salt bridges. The peptides also differ in their secondary structure (transient) motifs as well as in solvent exposure of hydrophobic residues (such as in the central hydrophobic cluster). We explain possible implications of these findings on propensity and pathways of aggregation, as well as on the structure of small Aβ oligomers and molecular mechanisms of their neurotoxicity.

P-036: Modulation of protein quality control pathways as a novel intervention strategy in prion diseases

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Increasing numbers of neurodegenerative disorders such as Alzheimer, Huntington's, Parkinson and prion diseases pose a tremendous disease and economic burden on our aging society. Prion diseases are prototypical protein misfolding diseases in human and animals caused by misfolding of the cellular prion protein (PrP^C) into the infectious PrP^{Sc} isoform. These diseases have the potential to transmit between or within species, including zoonotic transmission from animals to humans, and can result in serious endemic and epidemic scenarios. It is therefore critical to understand the molecular and cellular mechanisms of prion propagation and transmission to elucidate molecular strategies for disease control. Previous studies have demonstrated a direct influence of impairment in quality control mechanisms on the conversion of PrP^C into PrP^{Sc} and that over-expression of some quality control proteins can reduce prion conversion. In this study, we want to manipulate cellular quality control pathways *in vitro* and *in vivo* by stably over-expressing selected folding and sorting proteins in order to show that this represents a novel pathway counteracting prion propagation. The overall objective of our work is to study the cellular and molecular biology of prion infections and utilize it for delineating novel targets for intervention. We are using a cutting-edge lentiviral gene therapy technique to stably transfer therapeutic target genes into prion-infected cells and animal models and determine its effect on prion conversion. The candidate genes are those encoding the proteins

involved in proteasomal degradation (EDEM1, EDEM2 and EDEM3), folding (ERp57) and secretory protein cargo transport (ERGIC-53 and VIP36). In addition, prion-infected cells are transiently transfected with vectors expressing target genes to evaluate their effect on PrP^{Sc} levels. The overall outcome of this work will provide new mechanistic insights into prion infections and define a novel therapeutic strategy against prion diseases and protein misfolding disorders.

P-037: Effect of substitutions equivalent to bank vole 109I polymorphism in the spontaneous misfolding ability of PrPs from several mammalian species

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Although the large majority of human TSE cases are from sporadic or genetic origin, meaning that PrP^{Sc} is spontaneously generated in the central nervous system, most of the experimental models used require an exogenous infectious agent in order to recapitulate all the hallmarks of prion diseases. However, this scenario has recently changed due to a new animal model that can hide some answers about sporadic TSE development. The description of the bank vole as a highly susceptible species to prions from several species *in vivo*, ex vivo and *in vitro* drew the attention of many researchers and led to the generation of a transgenic mouse line that overexpresses a polymorphic bank vole PrP (109I). Surprisingly, it induces spontaneous and transmissible prion disease in contrast to the bank vole 109M PrP, being the first animal model that develops a sporadic and transmissible prion disease in the absence of mutations in PrP.

Using PMCA based on recombinant PrP as substrate (recPMCA), we intended to shed some light on the effect of the 109I polymorphism and the spontaneous misfolding ability of bank vole PrP. After successfully achieving to replicate spontaneous misfolding of bank vole 109I PrP in unseeded serial recPMCA, we studied whether this polymorphism can exert the same effect in other species. For that purpose, we chose mouse PrP to perform L108I substitution and test its spontaneous misfolding ability *in vitro* and *in vivo*. Given the observation that it misfolds spontaneously as bank vole 109I PrP, we wondered how would any other substitution in the same position affect the misfolding ability of the mouse PrP. Unexpectedly, few amino acids from all the possible variants tested in position 108 conferred spontaneous misfolding ability *in vitro*, although the 108I showed the strongest effect. The next question that arose was whether this polymorphism could act similarly in phylogenetically more distant mammalian species. Thus, positions equivalent to bank vole 109 were substituted for isoleucine in human, sheep, cattle, mule deer and few other species PrP and their spontaneous misfolding ability as well as transmissibility to wild type (wt) and other species of interest was tested by recombinant PMCA. Surprisingly, 109I equivalent substitution conferred not just spontaneous misfolding ability to most of the PrPs studied but also the *in vitro* misfolded PrPs were able to propagate in their wt counterparts and also in other species, showing the strong connection of this position with sporadic transmissible prion generation.

P-040: Cellular phenotypes of prion disease in skin-derived fibroblasts of asymptomatic PrP mutation carriers and sporadic CJD patients

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The conversion of a protease-sensitive cellular prion protein (PrP^{C}) into the pathological insoluble protease-resistant isoform (PrP^{Sc}) is the key molecular event in the pathogenesis of prion diseases. Cell models are critical for us to understand the cellular events associated with the spontaneous PrP^{C} to PrP^{Sc} conversion. Indeed, a PrP^{Sc}-like form has been observed in human cell models; but these cells are not ideal since they are virtually all of cancer origin and their mutant PrP molecules are artificially transfected. Moreover, there have been no cell models available for study of sporadic Creutzfeldt-Jakob disease (sCJD). Here, we report the generation of skin-derived fibroblasts from 24 patients including 4 sCJD patients, 10 asymptomatic PrP mutation carriers with 6 different PrP mutations, and 10 non-CJD controls. We characterized the physicochemical properties of PrP and the cellular phenotypes in these patient-specific fibroblasts. As observed in the brain, mutant PrP glycoform is also different from that of wild-type PrP in the fibroblasts. Furthermore, protease-resistant PrP, the biomarker of prion disease, is also detectable in fibroblasts from some of the asymptomatic PrP mutation carriers and sCJD.

P-041: Gerstmann-Sträussler-Scheinker diseases with P102L, A117V and F198S mutations transmit efficiently and produce distinct pathological phenotypes in bank voles

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Gerstmann-Sträussler-Scheinker disease (GSS) is an inherited neurodegenerative disorder associated with mutations in the human PrP gene (*PRNP*). It is characterized by atypical PrP^{Sc} with an internal protease-resistant core (PrP^{res}) of 6-8 kDa. Attempts to transmit GSS in rodents have showed that only the few cases accompanied by 21 kDa PrP^{res} were transmissible, leading to the hypothesis that GSS exclusively associated with the internal PrP^{res} are non-transmissible proteinopathies rather than true prion diseases.

We recently reported the efficient transmission of GSS cases with P102L (n = 3), A117V (n = 2) and F198S (n = 2) mutations in bank vole (*M. glareolus*), carrying isoleucine at codon 109 of PrP (Bv109I). Notably, all GSS inocula induced the accumulation of internal PrP^{res} fragments, although a P102L case with 21 kDa PrP^{res} led also to the accumulation of 21 kDa PrP^{res} in some recipient voles. Overall, we observed 3 different PrPres types in voles that seemed to correlate with different survival times, suggesting specific biological properties. Indeed, the distinct disease phenotypes observed in inoculated voles at first passage

were consistent with prion strain variation upon subpassages, which led to the isolation of 3 different vole-adapted GSS-derived prion strains. Two of these strains were characterized by the accumulation of internal PrP^{res} fragments, although with distinct molecular weight of 7 kDa or 8 kDa; another vole-adapted strain was accompanied by 21 kDa PrP^{res}. Interestingly, a single vole-adapted strain was isolated from A117V and F198S GSS cases, while strain variation occurred among and within P102L GSS cases.

These findings imply that brains of GSS patients harbor transmissible prions, with features similar to those of classical prions, including strain variation. Furthermore, the existence of different strains of GSS might contribute to explain its marked clinical and pathological heterogeneity.

P-042: Identification of the origin of Creutzfeldt-Jakob disease after cadaver-sourced pituitary growth hormone treatment using an amplification property in protein misfolding cyclic amplification

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Background: Cadaver-sourced pituitary growth hormone (hGH) and dura mater-grafts represent the 2 most common causes of iatrogenic Creutzfeldt-Jakob Disease (CJD). Interestingly, amyloid plaques of the kuru type are often observed in both hGH-CJD and dCJD, even in cases with methionine homozygosity at codon 129 of the PRNP gene (129M/M). Transmission studies have shown that dCJD with amyloid plaques (p-dCJD) in Japanese carrying MM at codon 129 is caused by a prion strain originating from sporadic CJD (sCJD)-VV2 or -MV2 (V2 strains), which can be identified based on numerous kuru plaques and protease-resistant prion protein (PrP^{Sc}) intermediate in size between types 1 and 2. Recently, we developed protein misfolding cyclic amplification (PMCA) as a rapid diagnostic method for identifying acquired CJD associated with the V2 strain. In this study, we demonstrate that hGH-CJD shares the amplification characteristics associated with the V2 strain.

Methods: Seeds for PMCA reactions were prepared from autopsy human brain specimens from 5 hGH-CJD (3 cases: 129M/M, 2 cases: 129M/V), 4 different sCJD subtypes (MM1, MV2K, VV1, VV2), or p-dCJD. FreeStyle™ 293F cell lysates from a cell line stably expressing human PrP^{c} with methionine or valine at codon 129 were used as substrate for PMCA of human prions. A single round of PMCA (48 cycles of sonication and incubation) was carried out. All the samples were digested with proteinase K and an amplification factor was estimated by Western blotting.

Results and Discussion: Amyloid plaques were observed in all of the hGH-CJD cases by immunohistchemistry. Western blot analysis of PrP^{Sc} using 15% Bis-Tris gel revealed that the sizes of PrP^{Sc} in all of the hGH-CJD cases with the 129M/M genotype were in between type 1 PrP^{Sc} and type 2 PrP^{Sc} , resembling the intermediate-sized PrP^{Sc} found in p-dCJD cases. Although no amplification of sCJD-MM1 or -VV1 prions was observed with either the 129M or 129V substrate in PMCA, all samples from hGH-CJD were drastically amplified with the 129V substrates. Moreover, the PMCA products with the 129V substrates were identified as type 2 by using a type 2 PrP^{Sc} -specific

antibody. The amplification properties of these hGH-CJD are identical to those of p-dCJD, suggesting that these hGH-CJD cases resulted from a contamination of hormone preparations by a V2 CJD strain.

Reference: Takeuchi A et al. (2016) Distinctive properties of plaque-type dura mater graft-associated Creutzfeldt-Jakob disease in cell-protein misfolding cyclic amplification. Laboratory Investigation. in press.

P-044: Kinetics of RML prion propagation in 3 inbred mouse strains with indistinguishable expression levels of PrP^c but distinct incubation periods

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Prions are lethal infectious agents thought to consist of multi-chain forms (PrP^{Sc}) of misfolded cellular prion protein (PrP^c). Prion propagation proceeds in 2 distinct mechanistic phases: an exponential phase 1 which rapidly reaches a fixed level of infectivity irrespective of PrP^c expression level and a plateau (phase 2) which continues until clinical onset with duration inversely proportional to PrP^c expression level. We hypothesized that neurotoxicity relates to distinct neurotoxic species produced following a pathway switch when prion levels saturate. Indeed, we have shown a linear increase of proteinase K-sensitive PrP isoforms distinct from classical PrP^{Sc} at a rate proportional to PrP^c concentration, commencing at the phase transition and rising until clinical onset. Also, the unaltered level of total PrP during phase 1, when prion infectivity increases a million-fold, indicates that prions comprise a small minority of total PrP. This is consistent with PrP^c concentration not being rate limiting to exponential prion propagation.

It has been long established that inbred mouse lines with identical PrP alleles and indistinguishable levels of PrP^c expression have distinct incubation periods related to the effect of a number of modifier genes. We have now measured prion propagation and levels of disease-associated PrP prion levels in NZW, C57Bl-6 J/OlaHsd and CAST mice, 3 wild type strains showing substantially different incubation periods following RML infection, to understand how these genetic determinants of incubation period affect the phases of prion propagation we have described. We first confirmed that PrP^c expression levels in these lines were closely similar. Analysis of prion propagation after RML infection in these mice show the same 2 phase kinetics as seen previously. Interestingly, the exponential phase I is unaffected by the change in genetic background and the variation in incubation periods arises from differences in the length of the plateau phase. Further, we find that total PrP is essentially unaffected in phase 1 and that the increase in disease-related proteinase-K sensitive PrP again occurs in the plateau phase. These findings suggest that effects of the modifier genes may be on host sensitivity to neurotoxic species rather than on the kinetics of prion propagation.

This research was funded by the Medical Research Council.

P-045: A cellular bioluminescence assay detects prion protein dimerization and aggregation upon infection

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Prion diseases like Creutzfeldt-Jakob disease (CJD) are fatal neurodegenerative disorders that are triggered by misfolding of the cellular

prion protein (PrP^c) to an infectious isoform rich in β-sheet structure termed PrP^{Sc}. Because PrP^c forms dimers, dimerization of PrP^c may be an essential intermediate step for the formation of PrP^{Sc} aggregates upon infection.

To study PrP dimerization, and PrP aggregation upon infection, we developed a bimolecular complementation assay using RK13 cells that lack endogenous PrP expression and stably express 2 fusion constructs between PrP and each half of split Gaussia luciferase. In these cells bioluminescence occurs when PrP dimerizes or aggregates and the 2 split luciferase halves complement each other.

Using a panel of 8 antibodies directed against different PrP domains, we identified the central domain of PrP^c as critical for dimerization. Divalent cations were not essential for PrP^c-dimerization. Unlike normal brain homogenate infection of RK13 cells with brain homogenates from diseased animals containing 6 different mouse-adapted prion strains, RML, 22L, ME7, 87V, 79A, and 22A, lead to an increase in bioluminescence. In contrast, treatment of chronically infected but not uninfected RK13 cells with the anti-prion compound quinacrine reduced bioluminescence. Finally, screening of a compound library with 1650 bioactive compounds allowed us to identify compounds that inhibit PrP^c-dimerization. Treatment of ScN2a cells with some of these compounds reduced PrP^{Sc} levels in these cells at nanomolar concentrations.

Our results show that PrP^c clearly dimerizes in cells and that prion infection leads to increased bioluminescence in this cell assay. We identified bioactive compounds that inhibit PrP^c-dimerization and reduce PrP^{Sc} levels in ScN2a cells at nanomolar concentrations suggesting that PrP^c dimerization may be a critical step in the conversion of PrP^c to PrP^{Sc}.

P-046: Altered dynamics of membrane microdomain distribution in chronically prion-infected cells

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Prion diseases are characterized by extraneuronal deposits of disease-associated PrP (PrPd), spongiform alterations and rapidly progressing neurodegeneration. According to the protein-only hypothesis the prion protein (PrPc) is converted by template-assisted conformational changes to aggregation-prone β-sheet rich conformers. In neuronal cells, PrPc is expressed at the plasma membrane where it segregates into cholesterol-rich rafts. It has been suggested that protein aggregation in membrane microdomains may act as a general sorting signal to remove potentially noxious proteins, but the underlying molecular mechanisms are unknown.

We are using superresolution microscopy techniques, like Structured Illumination (SIM) and Stochastic Optical Reconstruction microscopy (STORM) to study the localization and membrane environment of PrP conformers in hyper-infected and uninfected cells. To characterize the distribution of raft and tetraspanin-containing microdomains, we labeled cells with the raft marker Cholera Toxin subunit B (CtxB) and with antibodies against tetraspanin proteins, respectively.

Hyper-infected cells are characterized by a high proportion of PrP^{Sc}-positive cells (>95%) on Scrapie Cell Assay and showed abundant deposits of PrPd at the extracellular matrix (ECM). The appearance of Ctx clusters at the ECM was observed exclusively in hyper-infected cells, while in uninfected cells, Ctx primarily was mainly detected in the plasma membrane and endosomal compartments. Unexpectedly, PrPd also colocalised with tetraspanin-containing membrane microdomains at the ECM. In summary, this data suggests that protein aggregation leads to rerouting of protein and lipid microdomains.

P-047: A comprehensive study of the potential resistance of the canidae family to prion infection

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One of the characteristics of prions is their ability to infect some species but not others. Thus, prion resistant species have been of special interest in the field because of their potential in deciphering essential determinants for susceptibility.

Previously, our group has been involved in the development of different *in vitro* and *in vivo* models to assess the susceptibility of species that, as Leporidae and Equidae families, were erroneously considered resistant to prion infection.

Here, we show a similar *in vitro* and *in vivo* approach developed to understand the unprecedented low prion susceptibility of the family Canidae.

Initially, studies based on the amino acid sequence of the canidae PrP have allowed us to identify unique key amino acids whose characteristics could orchestrate its high resistance. To confirm this, cell- and brain-based PMCA studies among others have been performed highlighting the relevance of those amino acids. An *in vivo* confirmation study was performed through the generation of transgenic mouse models carrying the substitutions of interest. Surprisingly, these animals show a complete resistance after intracerebral challenge using several prion strains, corroborating the key importance of the selected substitutions.

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

P-048: Classification of anti-prion compounds based on the binding properties to prion proteins

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To date, a variety of anti-prion compounds have been reported that are effective in ex vivo and also *in vivo* treatment experiments. However the molecular mechanisms of most of these compounds remain unknown. Here we classified anti-prion mechanisms into 4 categories; I: conformational stabilization, II: interference with the interaction between the cellular form of prion protein (PrP^C) and the scrapie form (PrP^{Sc}), III: precipitation of prion proteins, and IV: interaction with proteins other than PrP^C.[1] To characterize the anti-prion compounds according to this classification, we determined their binding affinities to PrP^C and their binding sites in PrP^C using the surface plasmon resonance and NMR spectroscopy, respectively. GN8,[2] GJP49,[3] TGV[4] bind specifically to the hot spot in PrP^C, and act as 'medicinal chaperones' to stabilize the native conformation and interfere with the interaction between PrP^C and PrP^{Sc}. Thus mechanism I & II are predominant. While quinacrine and epigallocathechin bind to PrP^C rather non-specifically. They may mainly interfere with the intermolecular interaction, and mechanism II may be applied. RNA aptamer R12 binds specifically to the N-terminal region of PrP^C and

has anti-prion activity,[5] which is also categorized into the mechanism II. On the other hand Congo red and pentosan polysulfate bind to PrP^C and cause its precipitation reducing the effective concentration of prion protein. So, mechanism III is appropriate. Finally CP60, an edarabone derivative, FK506[6] etc. never bind to PrP^C but may interact with PrP^{Sc} or other relevant proteins, and can be explained by mechanism IV, whose details must be elucidated further. The proposed characterization of diverse anti-prion compound would help understanding their anti-prion activities as well as facilitating further effective anti-prion drug discovery.

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P-049: A platinum compound targeting the cysteine residues of disease-related form of prion protein in cell lysates

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In prion diseases, a β-sheet-rich disease-related isoform of PrP is transformed by an unknown mechanism from an α-helix-rich isoform. We have previously discovered a platinum-containing compound that removed PK-resistant PrP (PrPres) in not only prion-infected mouse neuroblastoma cells but also their cell lysates in a dose-dependent manner. In the cell lysates, the compound also eliminated free cysteine residue-containing proteins such as glyceraldehyde 3-phosphate dehydrogenase and β-actin, whereas it did not eliminate a large portion of PrPc which had an intramolecular disulfide bond and no free cysteine residues. In this study, we examined the molecular mechanism of this phenomenon by means of biochemical approaches, using recombinant mouse PrP (rPrP). Three types of rPrP were prepared: oxidized rPrP (rPrP-Ox), reduced rPrP (rPrP-Red), and N-ethylmaleimide-blocked rPrP (rPrP-NEM). The rPrP-Ox was prepared by an arginine and oxidized glutathione-assisted refolding procedure. The rPrP-Red was prepared from rPrP-Ox by incubation with reducing agent dithiothreitol (DTT) and was recovered in 10 mM sodium acetate (pH 3.1). For preparation of rPrP-NEM, free cysteine residues of rPrP-Red were blocked with N-ethylmaleimide. The reaction mixture containing 1 ug of rPrP and ~0.2 mM platinum compound was incubated in 100 uL of PBS containing of 0.5% NP-40, 0.5% sodium deoxycholate and 50 mM imidazole at 37C for 30 min. The reaction was stopped by addition of DTT at a final concentration of 50 mM. The mixture was mixed with SDS-PAGE sample buffer and then subjected to SDS-PAGE and immunoblot analysis. The results showed that the compound treatment decreased rPrP-Red signals, while it did not affect the signals of rPrP-Ox and rPrP-NEM. In addition, the compound treatment did not affect rPrP-Red signals when a low concentration of cysteine or reducing agent was present. Coomassie blue staining of SDS-PAGE gel revealed that the compound converted rPrP-Red into an insoluble form even in the presence of SDS. However, this insoluble rPrP was fully resolubilized by heating in a reduced condition. In the cell lysates, compound-induced insoluble PrP was also resolubilized in a reducing condition in the presence of protease inhibitors. These findings suggest that the compound targets the free cysteine residues of PrP and converts free cysteine residue-containing PrP into a structurally unstable form that is susceptible to endogenous proteases in the cell lysates but is insoluble even in the presence of SDS. The

findings imply the possibility of the presence of free cysteine residue-containing PrPres in the prion-infected cells.

P-050: A kinetic model of the aggregation of α-synuclein provides insights into prion-like spreading

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The protein α-synuclein self-assembles into small oligomeric species and subsequently into amyloid fibrils, that accumulate and proliferate during the development of Parkinson disease. However, the quantitative characterization of the aggregation and spreading of α-synuclein remains challenging to achieve. Previously, we identified a conformational conversion step leading from the initially formed oligomers to more compact oligomers preceding fibril formation. Here, by a combination of single-molecule fluorescence measurements and kinetic analysis, we find that the reaction in solution involves 2 unimolecular structural conversion steps, from the disordered to more compact oligomers, and then to fibrils, which can elongate by further monomer addition. We have obtained individual rate constants for these key microscopic steps by applying a global kinetic analysis to both the decrease in the concentration of monomeric protein molecules and the increase in oligomer concentrations over 0.5-140 micromolar range of α-synuclein. The resulting explicit kinetic model of α-synuclein aggregation has been used to quantitatively explore seeding the reaction by either the compact oligomers or fibrils. Our predictions reveal that, although fibrils are more effective at seeding than oligomers, very high numbers of seeds of either type, of the order of 10,000, are required to achieve efficient seeding and bypass the slow generation of aggregates through primary nucleation. Complementary cellular experiments demonstrated that 2 orders of magnitude lower numbers of oligomers were sufficient to generate high levels of reactive oxygen species, suggesting that effective templated seeding is likely to require both the presence of template aggregates and conditions of cellular stress.

(This study is currently in press with PNAS).

P-051: Proteomic screening and identification of a structural protein of [NSI+] prion determinant in yeast Saccharomyces cerevisiae

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Prions are self-perpetuating protein conformations usually manifested by infectious protein aggregates. Previously, we discovered [NSI+]: novel non-chromosomal determinant, which possesses main features of yeast prions including cytoplasmic infectivity, reversible curability, dominance, and non-Mendelian inheritance in meiosis [Saifitdinova et al., Curr. Genet., 2010, V.56, P.467-478]. This determinant was demonstrated to cause omnipotent nonsense suppression at the genetic background of different mutant alleles of SUP35 as well as inhibition of the vegetative growth [Nizhnikov et al., Curr. Genet., 2012, V.58, P.35-47].

Further, we carried out a set of genetic screenings to identify the structural gene of [NSI+], but all the attempts of traditional genetic identification were unsuccessful. For this reason, we developed a method for proteomic screening of amyloids and prions (PSIA)

[Nizhnikov et al., PLoS One, 2014, e116003], which is based on the resistance of amyloid oligomers to treatment with ionic detergents and consists of 3 main stages: (i) extraction and purification of detergent-resistant protein fractions, (ii) trypsinolysis of proteins and separation of peptides by high performance liquid chromatography (HPLC) followed by (iii) mass-spectrometric identification of separated proteins. We compared detergent-resistant protein fractions of [NSI+] and [nsi-] strains using PSIA and found that these fractions were different in 2 proteins, whose detergent-resistant oligomers were detected only in the [NSI+] strain: Rnq1 and Swi1. These proteins are known to form prions [PIN+] and [SWI+], respectively. Detailed analysis demonstrated that both [SWI+] and [PIN+] are responsive for the nonsense suppression in the [NSI+] strain: elimination of [PIN+] significantly decreases nonsense suppression in this strain, while elimination of [SWI+] results in the complete loss of the suppressor phenotype. Induction of [SWI+] leads to weak nonsense suppression in our system, while [PIN+] does not cause nonsense suppression itself, but drastically increases the suppressor effect of [SWI+]. Therefore, we demonstrated that interaction of prions similarly to interaction of classical genes may lead to heritable changes.

The study was supported by the grant of the President of the Russian Federation (Project MK-4854.2015.4), RFBR (Projects 16-34-60153 and 14-04-01463), SPbSU (1.50. 2543.2013). The authors acknowledge St. Petersburg State University for opportunity to use facilities of the Research Resource Center for Molecular and Cell Technologies.

P-052: Aggregation of QN-rich fragment of Gln3 in yeast Saccharomyces cerevisiae is modulated by [PSI+] and [PIN+] prions

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Gln3 is a regulator of nitrogen metabolism in yeast Saccharomyces cerevisiae. Amino acid sequence of this protein has a region rich in glutamine (Q) and asparagine (N) residues (Gln3QN). We compared the effects of overexpression of full-length Gln3 and its QN-rich fragment, Gln3QN, fused with yellow fluorescent protein (YFP). Overexpression of full-length Gln3-YFP causes strong growth defect and Gln3-YFP diffusely localizes in nucleus. In contrast to full-length Gln3, Gln3QN-YFP forms multiple fluorescent foci located in cytoplasm and does not affect vegetative growth. Aggregates of Gln3QN-YFP possess resistance to treatment with ionic detergent sodium lauryl sarcosinate. Formation of Gln3QN-YFP aggregates occurs even in the strains lacking yeast prions, but the frequency of the cells with aggregates strongly depends on the presence of [PSI+] and [PIN+] yeast prions. So, [PIN+] drastically increases percentage of cells with Gln3QN-YFP aggregates, while [PSI+] does not significantly affect aggregation of Gln3QN-YFP itself, but strongly decreases it in [PSI+] [PIN+] strains in comparison to [psi-][PIN+]. The analysis of colocalization of the aggregates of Gln3QN-YFP with the aggregates formed by the structural proteins of [PSI+] (Sup35) and [PIN+] (Rnq1) fused with cyan fluorescent protein (CFP) coincided the data discussed above. So, the aggregates of Gln3QN-YFP colocalize with Rnq1-CFP aggregates in the [PIN+] strains with the frequency near 100%, while the same for the aggregates of Gln3QN-YFP and Sup35NM-CFP (prion-forming

domain of Sup35 fused with CFP) in the [PSI+] strains barely reaches 50%. Overall, we may conclude that aggregation of Gln3QN-YFP is modulated by the interaction between 2 prions, [PSI+] and [PIN+], that provides a versatile model to study the mechanisms of interaction and cross-seeding of QN-rich prions.

The study was supported by the grant of the President of the Russian Federation (Project MK-4854.2015.4), RFBR (Projects 16-34-60153 and 14-04-01463), SPbSU (1.50.2543.2013). The authors acknowledge St. Petersburg State University for opportunity to use facilities of the Research Resource Center for Molecular and Cell Technologies.

P-055: Yeast-based search for new human amyloidogenic proteins

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Many neurodegenerative diseases are associated with accumulation of toxic, highly structured self-assembled protein aggregates, termed amyloids. Recently emerging evidence indicates that many amyloids possess transmissible (prion-like) properties. In yeast, endogenous prions transmit phenotypically detectable traits. Some amyloid-like and prion-like protein polymers have been linked to biologically positive phenomena. In vitro experiments suggest that many proteins possess amyloidogenic properties. However, formation and propagation of amyloids are difficult to investigate *in vivo* due to complexity of the human organism. Therefore, we have established a yeast model for studying prion properties of mammalian proteins. We have demonstrated that fusion of a mammalian amyloidogenic protein to the prion domain of the yeast prion protein Sup35 enables such a chimeric construct to nucleate a prion in the absence of any pre-existing prions in the yeast cell. Phenotypic and biochemical detection assays, previously developed for the Sup35 prion, can be applied to detection of prion nucleation and propagation by chimeric proteins.

Here, we apply this yeast prion nucleation assay to search for human proteins and protein domains with amyloidogenic properties. By using a computational approach ArchCandy, we have identified a set of human proteins which contain domains with a high predicted propensity of forming β-arches, a characteristic structural element of amyloids. At least some of these proteins were proven to nucleate a prion in our yeast assay. This indicates that human genome codes for some previously unknown amyloidogenic and prionogenic proteins. Potential biological implications of amyloid formation by specific proteins will be discussed. (This work is supported by the grant 14-50-00069 from Russian Science Foundation. The authors acknowledge the SPbSU Resource Centers, “CHROMAS” and “Molecular and Cell Technologies," for technical support).

P-056: Analysis of interspecies prion transmission in yeast

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Self-perpetuating cross-β aggregated proteins (prions) are associated with fatal transmissible spongiform encephalopathies in mammals (including humans) and control heritable traits in yeast and other fungi. Prion diseases, such as sheep scrapie, bovine spongiform encephalopathy (BSE), chronic wasting disease (CWD) and

human Creutzfeldt-Jakob disease, are transmitted by the prion protein (PrP) that converts normal cellular protein of the same sequence into a polymeric cross-β (prion) isoform. Prion transmission depends on the identity of interacting amino acid sequences, so that the transmission barriers are observed between divergent species. However, these barriers can be overcome. For example, BSE transmission to humans is a huge problem for the cattle industry and for a public health. The mechanisms of cross-species prion transmission are still poorly understood.

We used a yeast Sup35/[*PSI*+] model to explore the molecular basis of prion transmission barriers. In contrast to the previous experimental setting where transmission of specific prion strains was tested, we used approach of cross-species prion induction by overproduced protein, where multiple prion variants can be generated and transmitted. We studied the orthologs of the yeast prion proteins Sup35 from 4 different yeast species: *Saccharomyces cerevisiae*, *S. paradoxus*, *S. uvarum* and *Lachancea kluyveri*. These proteins from 60 to 90% of amino acid similarity in their prion domains in pairwise combinations. Computational approach ArchCandy was employed to compare the spectra of prion structures generated by divergent prion domains and to predict amino acid substitutions that can affect the inuitial prion formation and/or species barrier.

We have shown that all 4 Sup35 proteins form aggregates in *S. cerevisiae*, and that even proteins exhibiting species barrier are able to co-localize in the *S. cerevisiae* cells. By using Foerster resonance energy transfer (FRET) approach, we have demonstrated that colocalized heterologous proteins physically interact to each other, with highest efficiency of interaction observed in the most closely related combination of *S. cerevisiae* and *S. paradoxus*. As all pairwise combinations exhibit species barrier in prion transmission (albeith lower in case of *S. cerevisiae* and *S. paradoxus*, compared to other combinations), our data confirm that physical interaction between heterologous protein within a coaggregate is not sufficient for prion transmission.

This work was supported by the grants 14-50-00069 from Russian Science Foundation and 15-04-06650 from Russian Foundation for Basic Research. Authors acknowledge the SPbSU Resource Centers «CHROMAS» and «Molecular and Cell Technologies» for technical support.

P-057: Formation of a metastable stress-inducible prion by the yeast short-lived actin associated protein

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Amyloid-based prions are ordered protein aggregates associated with both devastating diseases and potentially adaptive traits. Amyloid formation *in vivo* is thought to result from alterations in protein homeostasis and cellular quality control system, however specific mechanisms remain elusive. Yeast prions are epigenetic determinants that alter different cellular processes and as a result of this significantly change the phenotype of the host. Thus prion mechanism offers a dynamic system for epigenetic regulation of phenotype in response to changing environment. Some non-prion amyloids are involved in adaptive processes. In yeast, prion formation is facilitated by a variety of environmental stresses including heat shock. How prions arise *in vivo* and by which mechanisms stressful conditions influence prion formation remains largely unknown. Yeast prion proteins usually contain glutamine (Q) and asparagine (N) - rich prion domains (PrDs) responsible for prion propagation. It is possible that the polymerization of a prion-forming protein results in formation of the initial prion "seed." This could be accelerated when the

misfolded prion protein is present at a high local concentration. Indeed, de novo formation of yeast prions is induced by transient overproduction of the prion-forming protein and is significantly enhanced by overproduction of nonhomologous yeast proteins with QN-rich domains. We show that Lsb2, a short-lived heat shock inducible yeast protein, forms a heritable aggregated state, [LSB+], possessing all the major characteristics of a prion and promoting prion formation by another protein, Sup35. Detergent resistant Lsb2 aggregates, are detected in the cultures overproducing Lsb2 and are maintained by the [LSB+] cells. Formation of detectable [LSB+] prion requires association of Lsb2 with the actin cytoskeleton. [LSB+] prion is mitotically unstable, but its stability is increased by a defect in Lsb2 ubiquitination or by substitution of the Lsb2 8Q stretch by 8N. By using the 8Q-to-8N derivative of Lsb2, we have demonstrated that formation of the [LSB+] prion is induced by heat shock. The acquisition of prion-inducing activity by Lsb2 can be traced to a single amino acid substitution that is absent in its paralog, Lsb1. Introduction of this substitution into Lsb2 approximately coincides with the acquisition of thermotolerance in the Saccharomyces clade. Our findings directly implicate the role of ubiquitin-proteasome system and actin cytoskeleton in formation of metastable transient prions influencing amyloid formation by other proteins and shed new light on protein-based inheritance mechanisms of protein assembly diseases.

Supported by grants R01GM09394 from NIH and MCB-1516872 from NSF.

P-058: Emergence and evolution of prion and prion-like proteins in the eukaryotic domain

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Prions in the budding yeast Saccharomyces cerevisiae propagate heritable phenotypes, uncover hidden genetic variation, function in large-scale gene regulation, and can act like diseases. Almost all these amyloid prions have asparagine/glutamine-rich (N/Q-rich) domains. Other 'prionogenic' proteins, have been shown to form amyloid *in vivo*, and to have N/Q-rich domains that can propagate heritable states in yeast cells. Also, there are >200 other S.cerevisiae proteins with prion-like N/Q-rich sequence composition. Furthermore, human proteins with such N/Q-rich composition have been linked to the pathomechanisms of neurodegenerative amyloid diseases.

We present results of evolutionary analysis of these proteins across fungi, and also across the whole eukaryotic domain. In fungi, we discover a huge emergence of N-rich prions/PAFs ancestors early in the budding yeasts Saccharomycetes (also called Hemiascomycetes); the ancestral sequences of many S. cerevisiae prion proteins also arose at the same time evolutionarily. Also, we discover evidence that selection pressures on N/Q-rich protein sequences against amyloidogenesis are not generally maintained in budding yeasts. Furthermore, we discuss how many human prion-like protein domains are deeply conserved across other animals, and correlate this with functional genomics data. These results help to delineate further the limits and ancestry of N/Q-rich prions.

P-059: Latent structural variation in a yeast prion monomer determines strain phenotypes

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A remarkable feature of neurodegenerative diseases is that mutations in causal genes or

discrete cellular conditions can trigger the formation of distinct amyloid conformations that can undergo alternate cellular physiological trajectories such as cytotoxicity or tissue specificity. However, how structural polymorphism in amyloid is formed from a monomeric protein is poorly understood. Here, we reveal the atomic basis for structural polymorphism in a monomeric protein and its physiological consequences for amyloid formation and cellular phenotype. Sup35NM, an N terminal fragment of Sup35, the protein determinant of [PSI+] prion yeast, can form 2 alternate amyloid core conformations. Using biophysical methods, we revealed that solvent-exposed residues are crucial determinants of alternate amyloid core regions. Long-range interactions in the prion domain allowed the formation of local compact structures, which regulate self-intermolecular interactions required for initiation of amyloid formation. The molecular cascade of structural determinism explained the existence of phenotypically distinct [PSI+] strains. Together, these results demonstrate that natively disordered protein monomers contain latent compact local structures that when disinhibited can drive alternate amyloid conformations, redirect chaperone-mediated fiber fragmentation and alter prion strain phenotypes. Therefore, conformational variation in intrinsically disordered monomeric proteins is a critical checkpoint for control of pathological outcomes by propagating amyloids.

P-060: Chaperone sorting factor Cur1 exhibits differential effects on yeast prions

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Cur1 is a protein sorting factor, which was discovered as a protein which cures [*URE3*], a prion form of yeast protein Ure2, when expressed at high levels. Cur1 is shown to aid in relocalization of chaperones between nucleus, cytosol and protein quality control (PQC) compartments. Previously, our screening for factors that enhance synthetic lethality of [*PSI*$^+$] (a prion form of the yeast release factor Sup35) in combination with mutations in another release factor gene (*SUP45*) led to identification of *CUR1* as a multicopy enhancer of [*PSI*$^+$] phenotype. Therefore, we studied effects of *CUR1* on [*PSI*$^+$] maintenance, in comparison to other prions. We have confirmed that excess Cur1 cures [*URE3*]. Surprisingly, we have observed that excess Cur1 strengthens and stabilizes different variants of [*PSI*$^+$] prion. Accordingly, deletion of *CUR1* slightly weakened strong [*PSI*$^+$]^S variant, while having no effect on weak [*PSI*$^+$]^W variant. We were unable to detect any influence of *CUR1* overexpression on *de novo* induction of [*PSI*$^+$]. However in the absence of either *CUR1* or its paralog *BTN2*, [*PSI*$^+$]^W prion variant exhibited increased loss after a short-term mild heat shock, compared to wild-type genetic background. We also produced a truncated derivative of the *CUR1* gene that exhibited stronger effects (compared to the wild-type gene) on

both [*URE3*] curing and [PSI^+] enhancement. Notably, the effects of both full-length and truncated Cur1 proteins on the prions were alleviated by coexpression of the Hsp40 chaperone Sis1 in a dosage-dependent manner. Fluorescence microscopy have shown that Cur1 overproduction led to Sis1 relocalization to nucleus in both [PSI^+] and [*URE3*] strains. These results suggest that the effects of Cur1 on yeast prions are due to changes in Sis1 localization, influencing [PSI^+] and [*URE3*] in the opposite ways. This work was supported by research grants from Saint Petersburg State University (15.61.2218.2013, 1.37.291.2015), RFBR (16-04-00202), and NSF (MCB-156872). Authors acknowledge technical support from the Resource Center Molecular and Cell Technologies of Saint Petersburg State University.

P-061: Modulation of yeast prion strain competition by host genetic background and molecular chaperons

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[PSI+] is the prion form of Sup35, a subunit of the yeast translation termination complex. When multiple [PSI+] strains co-infect a dividing host, only one prevails in the end. The host genetic background is involved in winner selection. In the 5V-H19 background, the VK strain dominated over the VL strain. The order of dominance was reversed in the 74-D-694 background. Chaperon proteins could also influence the outcome of strain competition. Overexpressing the Hsp70 chaperon Ssa1 or Ssa2 in 5V-H19 caused the VL strain to win out instead. Conversely, deleting SSA1 or SSA2 from 74-D-694 caused VK dominance. Expanding the Glycine/Methionine-rich domain of Sis1, an Hsp40 protein, also reversed the dominance relationship in 5V-H19, causing VL to flourish. However, swapping the polymorphic alleles of SSA1, SSA2 and SIS1 between 5V-H19 and 74-D-694, including cognate promoters, did not change the original background-specific dominance order. Our results suggest differential interaction of chaperon proteins with distinct strain conformations and indicate the existence of additional polymorphic cellular factors, which modulate [PSI+] propagation and are responsible for the genetic background effect. Analysis of the novel factors is presented.

P-062: Prophylactic efficacy of orally administered compounds on the progression of scrapie induced motor coordination deficits

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Transmissible Spongiform Encephalopathies (TSE's) are progressive and invariably fatal neurodegenerative diseases. Although there is no known cure, current therapeutic efforts target the conversion of the benign cellular prion protein (PRPC) to the harmful, infectious prion (PrPSC) and consequent cell death. Along these lines, we wanted to assess the prophylactic efficacy of orally administered, FDA approved compounds that have been shown to affect PrP conversion and/or cell death. Using a mouse model system of scrapie, we examined the effect of orally administered aspirin (ASA, 30 ug/ml ad libitum) or Tauroursodeoxycholic

Acid (TUDCA, 5% ad libitum) on motor coordination using the Rotarod behavior task. Mice were divided into the following groups: ASA or TUDCA pretreatment 2 weeks prior to inoculation, Scrapie, ASA or TUDCA intervention. All mice were trained on the Rotarod task prior to a single intraperitoneal injection (100ul of 5%) of ME7 inoculum (ASA study) or RML inoculum (TUDCA study). Once mice began ASA or TUDCA, they remained on it for the duration of the study. Mice were tested monthly on the Rotarod test. Motor coordination deficits were measured by the latency to fall off the Rotarod which accelerated from 4 to 40 rotations per minute over the course of 5 minutes. Our data indicate that scrapie mice but not ASA pretreated scrapie mice exhibited a motor coordination deficit at 197 d post inoculation (dpi). This difference was statistically significant (Bonferroni $t = 3.883$, $p < 0.01$). Subsequent data indicated that ASA pretreated mice did not begin to show motor coordination deficits until 276 dpi, a difference of 3−4 months. There was a bimodal peak in ataxia onset, which necessitated euthanasia, in scrapie and ASA intervention mice but not ASA pretreated mice. Survival in the ASA study at 307 dpi: 25% ASA pretreated, 50% scrapie, 37.5% ASA intervention. TUDCA pretreated female mice trended toward better performance during the training phase of the rotarod task, prior to RML inoculation. As of 80 dpi there are no differences in motor coordination between TUDCA pretreated and RML only mice. Taken together, the current findings of this study suggest that it may be possible to retard the behavioral progression of TSE in mice using orally administered FDA approved compounds.

P-063: We shall overcome prion diseases only by using both scientific and empiric findings or why honoring discovery and development of avermectins with 2015 Nobel Prize for physiology or medicine does not decrease their chronic toxicity especially neural degeneration increasing the susceptibility for prion diseases

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The English organic farmer Mark Purdey was not right with his hypothesis that copper deficiency, oversupply of manganese, oxidative stress by the compulsory warble fly eradication scheme using Phosmet (organophosphate) and reducing the temperature in rendering plants in the early 1980s had caused the emergence of BSE (bovine spongiform encephalopathy) in Great Britain. These circumstances first caused prions in the food chain.

Only the marketing introduction of Ivermectin (macrocyclic lactone) in 1982, the production of meat and bone meal without total extraction of fat containing Ivermectin, the use of animal fats in milk replacers containing Ivermectin and the residues by antiparasitic treatment with Ivermectin extremely increased the susceptibility of bovines for BSE with its characteristic symptoms.

The GABA receptor stimulating Ivermectin leads to neural hyper polarization. Smallest concentrations for a long time cause neural degeneration in the form of vacuoles and plaque formation in brainstem and cerebellum as well as damage to optic and sciatic nerve.

Prions must come from the gut to the brain. Dendritic cells take on prions and convey them to cells of the lymphatic system. Here a transfer to the peripheral nervous system via tunneling nanotubes is possible, but only if the nerve cells were damaged by Ivermectin. PrPc with its globular portion is part of the double membrane of the neuron, the N-terminus hovers like a tail over the membrane. Hyperpolarizing increases

the electrostatic pressure to the membrane, the docking site for PrP^{Sc} on the globular part of PrPc is readily accessible, PrP^{Sc}-PrPc-reaction is made possible, the flexible tail descends to the membrane, the neuron is destroyed and the conformational change is completed.

In South Sudan and Uganda Nodding Syndrome is a mysterious illness (similar to Chronic Wasting Disease) that affects the brains and nervous system of children, primarily between the ages of 5 and 15. Yet the World Health Organization (WHO) and Centers for Disease Control (CDC) are not fully sure what is causing the illness. Theories have linked the disease to Onchocerca volvulus (the organism that causes River Blindness). River blindness is treated twice a year with Ivermectin (Mectizan); the epileptic seizures are treated with Phenobarbital. Both medicaments together with alcohol cumulate their effects on the GABA receptor.

The relationships presented here should not be ignored any longer but urgently investigated by experimental research. You will overcome prion diseases only along with the poster award winner of PRION 2012 Amsterdam.

P-064: Low activity of complement in the cerebrospinal fluids of the patients with various prion diseases

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The aim of this study was to analyze the states of the activity and the levels of complement in cerebrospinal fluid (CSF) of the patients with various prion disease (PrDs). Our proteomic data highlighted the levels of 20 identified complement components in the CSF panel of sCJD were lower than that of non-PrD. The 50% of complement hemolytic activity (CH50) assays revealed a markedly lower activity of complement in the CSF panel of sCJD. The decreased levels of 3 key complement subunits, C3a/α, C4β and C9, in the pooled CSF of sCJD were verified by Western blots. Furthermore, the CH50 values in CSF of 136 sCJD, 39 gCJD, 22 FFI and 145 non-CJD were individually tested. Compared with the control of non-PrD, the CH50 value in the CSF specimens of various PrDs, especially in 3 subtypes of inherited PrDs, were significantly lower. Relationship analysis identified that the CH50 activity in the CSF was negatively associated with the protein 14-3-3 positive in the CSF.These results indicate a silent state of complement system in the CSF of the patients of PrDs.

P-067: The density of M cells in the epithelium overlying the Peyer's patches influences susceptibility to oral prion disease

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Many natural prion diseases are orally acquired. Following exposure via this route, the early replication of some prion strains upon follicular dendritic cells (FDC) in the Peyer's patches in the small intestine is important for the efficient spread of disease to the brain (termed neuroinvasion). However, for the prions to replicate upon the FDC within the Peyer's patches they must first cross the gut lumen. M cells are a unique population of cells within the epithelia overlying the Peyer's

patches which are specialized for the transcytosis of particles and microorganisms. Our previous data suggested M cells play an important role in the initial uptake and transfer of prions from the gut lumen into Peyer's patches. Since certain pathogens and inflammatory conditions can dramatically modify the status of M cells in the gut epithelium, is it plausible that such changes may also influence oral prion disease susceptibility. In Peyer's patches stimulation by the cytokine RANKL induces the differentiation of enterocytes into M cells and maintains them in their differentiated state. Therefore, in the current study we manipulated RANKL-stimulation in the gut epithelium to modify M-cell density and studied the effects these changes had on susceptibility to oral prion infection. Mice in which RANK (the receptor for RANKL) is deleted only in the gut epithelium are deficient in M cells and unable to sample particulate antigens from the lumen. We show that in the specific absence of M cells in these mice the uptake of prions from the gut lumen and their replication upon FDC in Peyer's patches are likewise blocked. Conversely, exogenous administration of recombinant RANKL enhances M-cell differentiation within the gut epithelium and significantly enhances the uptake of particulate antigens into Peyer's patches. We also show that recombinant RANKL-mediated induction of M cells enhanced the early uptake of prions into lymphoid tissues and significantly reduced disease susceptibility. Together these data demonstrate that the density of M cells in the epithelia overlying the Peyer's patches in the small intestine has a direct influence on the uptake of prions from the gut lumen and disease susceptibility: in the specific absence of M cells prion disease susceptibility is blocked, whereas their increased density increases disease susceptibility. These data have important implications for our understanding of how changes to the gut epithelium may influence the risk of prion infection.

P-068: Challenging the central hypothesis that misfolded prion protein accumulation, spread and distribution predicts regions of neurodegeneration

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Protein misfolding is common across many neurodegenerative diseases, with misfolded proteins acting as seeds for "prion-like" conversion of normally folded protein to abnormal conformations. However, the mechanism(s) leading to neurodegeneration remain unclear. A central hypothesis is that misfolded protein accumulation, spread and distribution predict regions of neurodegeneration. We examined this hypothesis using a highly sensitive assay system (RT-QuIC) for detection of misfolded protein seeds in a murine model of prion disease. Misfolded prion protein was observed widespread throughout the brain, accumulating in all brain regions examined irrespective of neurodegeneration. Importantly, neither time of exposure nor amount of misfolded protein seeds present determined regions of neurodegeneration. We further demonstrate evidence of 2 distinct microglia responses in prion infected brains: (i) a novel homeostatic response observed in all brain regions tested, but which predominates in brain regions which do not undergo neurodegeneration, and (ii) an innate immune response restricted to sites of neurodegeneration. Our data shows that accumulation of misfolded prion protein, alone, does not define neurodegenerative targeting, which instead results only when misfolded prion protein accompanies a specific microglial-driven innate immune response.

P-071: Impairment of protease activated receptors calcium signaling in prion infected cell lines

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Introduction. Prion diseases are fatal neurodegenerative disorders accompanied by progressive neuronal impairment and accumulation of misfolded forms of the prion protein. The precise mechanism of neurodegeneration in prion diseases remains to be elucidated. We have previously observed modest but highly significant prolongation of survival of protease activated receptor-2 knockout mice (PAR2 -/-) after intracerebral inoculation with RML prions.[1] In this study we have investigated the effect of prion infection on protease activated receptors PAR1 and PAR2 calcium signaling in prion propagating cell lines.

Material and methods. Cell lines PK1 (clone of murine neuroblastoma N2a cells), CAD5 (clone of murine Cath.a differentiated cells) and SMB.s15 (murine mesodermal cell line) were included in the study. In CAD5 and PK1 cells which do not express functional PAR2, we provided the expression from transgene using lentiviral vector. Cell lines were infected with various doses of mouse adapted scrapie prions (RML and Chandler). The level of infection was determined by standard scrapie cell assay and western blot. Kinetics of intracellular calcium changes in response to various PAR1 or PAR2 agonists were measured by CAL520 calcium indicator using Victor3 plate reader (Perkin Elmer).

Results. The infection of cells led to decreased calcium response to both PAR1 and PAR2 stimulation. The decrease in calcium upregulation was proportional to the level of prion infection confirmed by standard scrapie cell assay and correlated with the decreased levels of cell intracellular calcium stores.

Conclusion. Our results demonstrate the impairment of cell calcium signaling after PAR1 or PAR2 stimulation in studied prion infected cell lines. However, the defect is probably linked to the diminished stores of intracellular calcium in the infected cells and not to the functional defect of the PAR1 and PAR2 signaling pathways.

Funding. The study was funded by grants of the Czech science foundation GACR P303/12/1791 and of the Charles University in Prague PRVOUK-P24/LF1/3.

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P-072: Defining the microglia response during the time course of chronic neurodegeneration

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Inflammation has been proposed as a major component of neurodegenerative diseases, although the precise role it plays has yet to be defined. We examined the role of key contributors to this inflammatory process, microglia, the major resident immune cell population of the brain, in a prion disease model of chronic neurodegeneration. Initially, we performed an extensive reanalysis of a large study of prion disease, where the transcriptome of mouse brains had been monitored throughout the time course of disease. Our analysis has provided a detailed classification of the disease-associated genes based on cell type of origin and gene function. This revealed that the genes upregulated during disease, regardless of the strain of mouse or prion protein, are expressed predominantly by activated microglia. In order to study the microglia contribution more specifically,

we established a mouse model of prion disease in which the 79A murine prion strain was introduced by an intraperitoneal route into BALB/cJFms-EGFP/ mice, which express enhanced green fluorescent protein under the control of the c-fms operon. Samples were taken at time points during disease progression, and histological analysis of the brain and transcriptional analysis of isolated microglia was carried out. The analysis of isolated microglia revealed a disease specific, highly proinflammatory signature in addition to an upregulation of genes associated with metabolism and respiratory stress. This study strongly supports the growing recognition of the importance of microglia within the prion disease process and identifies the nature of the response through gene expression analysis of isolated microglia.

P-073: The route of inoculation of kuru during traditional mortuary feasts in the kuru-affected region

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In this presentation we present a synthesis of new ethnographic data, collected during a collaboration between the Medical Research Council Prion Unit (United Kingdom) and the Papua New Guinea Institute of Medical Research, and epidemiological data on the wide range of incubation period and the age distribution in kuru to establish if the primary means of inoculation of kuru was via the oral or parenteral route during traditional mortuary feasts held in the kuru-affected region in the eastern highlands of Papua New Guinea. This question assumed importance because of the dietary exposure of populations to bovine spongiform encephalopathy and its implications for world-wide public health. Some early kuru investigators had previously emphasized parenteral inoculation as the primary or exclusive means of transmission of kuru over oral inoculation.

Although parenteral inoculation may have occurred in some cases the new investigations established that oral transmission during traditional mortuary rites was the primary route of inoculation of kuru. This conclusion followed from the long incubation periods and their wide range found in kuru, which is characteristic of oral but not parenteral transmission of prions, and the new ethnographic data that excluded parenteral transmission in children, who constituted a significant proportion of kuru cases.

P-074: New insights in the transfusional risk assessment of variant Creutzfeldt-Jakob Disease: Transfusional transmission of vCJD prions in the absence of detectable abnormal prion protein

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Twenty years after the onset of the first cases of variant Creutzfeldt-Jakob disease (vCJD), the apparent rate of transmission of Bovine Spongiform Encephalopathy (BSE) to human fortunately remains very low. However, according to recent British large scale retrospective prevalence studies based on appendix samples, 99% of infected consumers would remain asymptomatic and unidentified to date:

these healthy vCJD carriers would represent a prevalence of 1/2,000 in UK which constitute a risk, notably for blood transfusion, whose long-term impact cannot be quantified today.

In two models of conventional mice and in the cynomolgus macaque, the transfusion of infectious blood products transmitted the expected vCJD disease in a faint proportion of recipients, whereas 2- to 7-fold more animals developed fatal, neurological symptoms in the absence of detectable accumulation of proteinase K-resistant prion protein (PrPres) in brain, but presented pathognomonic lesions notably centered on the spinal cord. We also demonstrated that the deleucocytation of blood components had no effect to prevent the transmission of these new phenotypes that escape classical prion identification criteria. Similar unexpected phenotypes, which are transmissible and can induce a vCJD phenotype after secondary transmission, were also observed but in lower proportion when soluble brain infectivity was intravenously administered in the same models.

We showed 20 y ago that transmission of BSE might occur in the absence of prion specific lesions or PrPres deposition after intracerebral transmission of BSE in conventional mice; here we show that the intravenous route could similarly induce an incomplete disease phenotype with a preferential involvement of the spinal cord, which is a CNS area that is rarely investigated for the diagnosis of prion diseases. Our results question the usefulness of the current criteria for vCJD diagnosis in the case where a similar phenotype emerges in humans and, subsequently, the potential underestimation of the impact of BSE/vCJD prion in the exposed populations.

P-075: Neuronal toxicity of the expression of human PrP with the E200K mutation in the mecanosensitive neuronal system of the nematode C.01

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The activity of cellular prion protein (PrPc) involves a variety of ligands and signal pathways. The study of PrP molecules carrying pathogenic mutations may provide insight into the mechanisms of prion neurotoxicity and their link to the propagation process. Currently, this phenomenon remains poorly understood and only a few simplified genetical models are available. Caenorhabditis elegans is a nematode with a well-defined neuronal system (neurons lineage, neuronal connexions, neuro-transmitters. . .) showing a strong gene homology with mammals including humans, devoid of human PrP homolog, and has proven to be a powerfull genetical model to study the molecular mechanisms of cell toxicity notably due to a gain of function mechanism.

In a previous study, we showed that C. elegans model is appropriate to study the impact of mutated PrP on neuronal cell function. We generated transgenic worm lines expressing human PrP with an insertional mutation that produced an abnormal PrP sharing cellular and biochemical properties with that observed in patients with the same pathogenic mutation. Here, we expressed human PrP with the most frequent PrP mutation (E200K), which is responsible for inherited Creutzfeldt-Jakob disease, in the mechanosentive neuronal system of the nematode. This system allows the detection and the quantification of neuronal dysfunction by assessment of the motility of the worms induced by the stimulation of the mecano-sensitive reflex.

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

Here, we provide the first *in vivo* simplified model showing a prion-induced neuronal death. Our results validate the nematode C. elegans as a suitable model to study the molecular mechanisms of cellular dysfunction induced by mutant prion proteins. It will help to better understand the relationship between PrP aggregation and toxicity, some aspects of prion propagation and to identify molecules that may protect neuron from the detrimental effects of misfolded PrP con-formers.

P-076: Impact of human prion proteins with E211D or E211Q mutation on tau alterations in cultured cortical neurons

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Alzheimer disease (AD) and Gerstmann-Sträussler-Scheinker (GSS) syndrome share common neuropathological lesions: extracellular accumulation of amyloid proteins (either A-β peptides or PrPsc) associated with intra-neuronal aggregation of hyperphophorylated tau protein forming neurofibrillary tangles and neuropil threads. Neuritic plaques can be observed in both diseases. They are characterized by an amyloid core surrounded by tau-positive dystrophic neurites.

To identify factors that may contribute to the relationship between amyloid and tau pathologies, we took advantage from mutations at codon 211 of PRNP (Peoc'h et al., Hum Mol Genet. 2012). The substitution of glutamate (E) at codon 211 with either glutamine (Q) or aspartate (D), leads to distinct disorders at the clinical, neuropathological and biochemical levels (Creutzfeldt-Jakob disease (CJD) or GSS). When GSS was observed (E211D), a C-terminal truncated fragment of PrP was the major PK-resistant amyloid component detected in the affected brain. Results from molecular dynamics simulations and structural studies of mutant recombinant proteins provided evidence that each E211 substitution impacts differently the stability of the protein and its ability to form oligomers. Interestingly, in GSS patients, in addition to the classical prion related pathology, we observed abundant tau fibrillar pathology, similar to that observed in AD, although the amyloid core was composed of abnormal PrP instead of A-β peptides.

In this study we assessed the effect of PrP assemblies on tau alteration, using primary cortical neuronal cultures from transgenic mouse lines with various levels of PrP expression. The effect of cell exposure to monomers and protein assemblies formed by recombinant human PrPs (wild type, E211D and E211Q) on tau phosphorylation and cell viability was assessed.

We showed that exposure to the different assemblies of recombinant PrP do not induced detectable neuronal loss regardless of the PrP mutation (exposure to a concentration $\leq$100 μg/mL). The exposure to oligomeric assemblies of PrP had a significant impact on abnormal phosphorylation of tau unlike the exposure to monomeric solution. The increase

of tau hyperphosphorylation was particularly enhanced after exposure to E211D-PrP. Furthermore, this effect of PrP assemblies on tau alteration was significantly enhanced in neurons overexpressing cellular PrP.

Our results showing structure-dependent and mutation-specific effect of recombinant PrP on tau phosphorylation modulated by PrPc expression level are very consistent with what has been observed in E211D/Q patient brains. They provide a useful *in vitro* model to study PrP-tau interplay and pave the way to future studies aiming to better understand the cellular pathways involved in amyloid-induced tauopathies.

P-077: Is sporadic CJD an acquired disease? A review of the UK CJD cases

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It is believed that sporadic CJD is caused by a spontaneously occurring protein misfolding event, but the possibility of person-to-person transmission cannot be ruled out. Other forms of CJD have been transmitted between individuals, including through the usage of cadaveric-derived human growth hormone, dura mater grafts, and - in variant CJD - through blood transfusion. A number of epidemiological studies have also suggested surgery as a risk factor for sporadic CJD. It is possible, therefore, that at least some cases of sporadic CJD may in fact be acquired through medical or surgical intervention. We explore the UK sporadic CJD patient population for any evidence of linkage between cases to try to identify potential transmission.

All suspected CJD cases in the UK are referred to the UK National CJD Research & Surveillance Unit, for neurological and, where appropriate, pathological review. A detailed medical and surgical history is collected from the family using a standardised questionnaire, and this is supplemented by a further review, based on hospital case notes and the general practitioner (family doctor) medical records. Details of any blood transfusion, blood donation, tissue or organ transplantation, or surgery is collected from all data sources. For blood and blood products, the case details are passed to the UK blood services as part of the ongoing Transfusion Medicine Epidemiological Review (TMER); recipients of blood from, and donors of blood to CJD patients are identified and cross referenced with the national surveillance database of CJD patients in order to identify links between cases. To date there is no evidence of blood transfusion related transmission of sporadic CJD in the UK.

In patients suspected or known to have had tissue or organ transplantation, we will perform a lookback study to identify links between donors and recipients. For other types of surgery, the medical histories will be analyzed to look for patients undergoing related surgeries in the same hospital within a short time frame. Any identified links might indicate iatrogenic transmission of sporadic CJD and will be explored to consider this possibility. The results for tissue transplantation and surgery will be presented.

P-078: Subcutaneous administration of Mouse recombinant prion protein resistant or RML trigger long-term alterations in composition of intestinal microbiota in FVB/N female mice

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The objective of this study was to evaluate the effect of 1x subcutaneous (sc) administration of RML scrapie prions (PrPsc) and

proteinase K resistant recombinant mouse prion protein fibrils (PrPres) on microbiota composition in the small intestine and colon of FVB/N female mice at 735 dpi. moPrPres is a β-sheet rich isoform resistant to proteinase K digestion generated by incubation of recombinant moPrP with lipopolysaccharide. Forty-five FVB/N female mice at 35 d of age were randomly assigned to 3 treatment groups: 1) saline (control - CON), 2) moPrPres (29-232), and 3) RML. All three groups were administered saline (0.9% NaCl) sc at 0.11 μL/h using ALZET® osmotic mini pumps for a period of 6 wk. A simultaneous 1x sc injection of moPrPres (29-232; 45 μg/mice) or RML (107 ID 50 units of RML scrapie prions) was given at the time of minipump implantation to the designated treatment groups. All mice were euthanized 735 dpi. A mixture of gut contents from the intestines and the colon were collected for microbiota analysis. DNA isolation from the gut contents was performed using QIAamp DNA stool mini kit according to the manufacturer's protocol. Results of this study showed that both moPrPres and RML lowered the percentage of Firmicutes from 89% (CON) to 45.4% (moPrPres) and 41.1% (RML). Both moPrPres and RML also increased the percentage of members in the Bacteroidetes phylum from 8.5% (CON) to 28.3% (moPrPres) and 58.5% (RML). At the taxonomic family level both moPrPres and RML lowered the abundance of Lactobacillace compared to CON mice (87.7% vs 42.2% vs 27.4%, respectively). At the taxonomic family level moPrPres increased the abundance of Enterobacteriaceae and Sphingomonaceae compared to CON (0.9 vs 17.8% and 1.5 vs 6.0%, respectively), whereas RML increased the abundance of Lachnospiraceae, Rikenellaceae, and Bacterioidaceae compared to CON mice (0% vs 5.6%; 0% vs 5.6%, and 0% vs 18.1%, respectively). Results also showed that Lactobacillus was the most negatively affected genus by both moPrPres and RML versus CON mice (87% vs 42.2% vs 27.4%, respectively). Moreover, RML treatment significantly increased members of the genus Bacteroides and Alistipes (18.1% and 5.6% vs CON 0%), respectively. moPrPres also increased the percentage of the genus Shigella vs CON animals (0.8% vs 17.6%, respectively). In conclusion, 1x sc administration of moPrPres and RML caused similar long-term alterations in the intestinal microbiota composition by lowering the percentages of Firmicutes and increasing those of Bacteroidetes.

P-080: Early age oral administration of mouse recombinant prion protein resistant or RML trigger lifelong modifications in the composition of intestinal microbiota in FVB/N female mice

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The objective of this study was to evaluate the effect of oral administration of moPrPres (proteinase K resistant recombinant mouse prion protein) and RML PrPsc (Rocky Mountain Laboratory scrapie strain). The moPrPres was generated by incubation of moPrP (29-232) with Escherichia coli 0111:B4 lipopolysaccharide to generate a β-sheet rich, proteinase K resistant isoform. Thirty FVB/N female wild type mice (n = 10/group) were randomly assigned to 3 treatment groups and treated with: 1) oral spray of saline (0.9% NaCl) at 0.1 microgram/g BW for 3 wk, twice per wk; 2) oral administration of similar doses of saline twice per wk for 3 wk followed by one time oral administration of PrPres; 3) oral administration of similar doses of saline twice per wk for 3 wk followed by 1x inoculation of 10 to the 7 ID 50 units of RML scrapie prions. Five mice per group were euthanized at 147 dpi and the other 5 were left to develop disease and

terminated at 250 dpi. A mixture of gut contents from the intestines and the colon of mice terminated at 250 dpi were collected for microbiota analysis. DNA isolation was performed using QIAamp DNA stool mini-kit according to the manufacturer's instructions. Results showed that moPrPres and RML influenced the composition of intestinal bacteria compared with the CON group. In CON mice most of the classified sequences belonged to the Firmicutes (54.4%), Bacteroidetes (32.1%), and Proteobacteria (13.4%) phyla. In the moPrPre the phylum Firmicutes increased to 76.2%, whereas Proteobacteria and Bacteroidetes decreased to 8.8% and 14%, respectively. In the RML treated mice the dominant phyla were Firmicutes (97.6%), Actinobacteria (1.1%), and Bacteroidetes (0.8%). Moreover, the most abundant genera in the CON and moPrPres groups were Lactobacillus and Sphingomonas (CON: 43.2%, and 13.4%, respectively) and (moPrPres: 22.5%, and 8.7%, respectively). In the RML group, the genus Lactobacillus was most abundant, consisting of 68.1% of all genera. The fraction of unidentified genera in the 3 groups ranged from 20% to 40% of the OTUs. Another interesting observation was that family Lachnospiraceae remained similar between the CON and RML groups of mice (7.7% vs 7.0%); however, they increased in the moPrPre group to 16.5%. To the best of our knowledge this is the first report to demonstrate that oral moPrPres and RML trigger lifelong modifications in the composition of intestinal microbiota in FVB/N female mice that might contribute to pathobiology of prion disease.

P-081: Importance of complement receptor CD21 in establishing peripheral prion accumulation

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Common pathogenesis of transmissible spongiform encephalopathies (TSEs) after exposure involves prion accumulation first in peripheral lymphoid organs prior to neuroinvasion. Complement, a major component of the innate immune system, is vital for establishing accumulation of PrPRES. When complement is activated, cleaved products C3 and C4 differentially activate complement receptors CD21/35 (CR2/CR1) expressed on various cells of the immune system, including B cells and follicular dendritic cells (FDCs). CD21 and CD35 are alternatively spliced transcripts of the Cr2 gene in mice. Although signaling through CD21/35 strengthens innate and adaptive immune responses, it paradoxically exacerbates prion pathogenesis. Furthermore, elimination of CD21/35 reduces prion replication and disease. Naturally, the question of which splice variant (CD21 versus CD35) is more important in prion pathogenesis surfaces. In this study, we examined the relative importance of CD21 vs. CD35 in establishing prion infection in the spleen. We assessed prion accumulation in the spleens of CD21 knockout (CD21-/-) vs. CD35 knockout (CD35-/-) mice injected with 1% RML5 (Rocky Mountain Laboratory Strain, Passage 5) 30 d post inoculation (DPI). A semi-quantitative prion amplification scoring system based on protein misfolding cyclic amplification (PMCA) was then used to compare the 2 gene variants. Our data shows CD21 is more important than CD35 as CD21-/- accumulated significantly less prions in the spleen. Furthermore, CD21-/- mice survived significantly longer than CD35-/- mice in a terminal study. These findings not only highlight a distinction in prion pathogenesis between CD21 and CD35, but they also further illuminate the importance of certain cell types in establishing prion disease.

P-083: Prion strain-dependent effect of macroautophagy on abnormal prion protein degradation

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Prion diseases are neurodegenerative disorders caused by the accumulation of abnormal prion protein (PrP^{Sc}) in the central nervous system. With the aim of elucidating the mechanism underlying the accumulation and degradation of PrP^{Sc}, we investigated the role of autophagy in its degradation, using cultured cells stably infected with distinct prion strains. The effects of pharmacological drugs that inhibit or stimulate the cellular signal transduction pathways that mediate autophagy during PrP^{Sc} degradation were evaluated. The levels of PrP^{Sc} in cells persistently infected with the prion strain Fukuoka-1 (FK), derived from a patient with Gerstmann-Sträussler-Scheinker (GSS) syndrome, was significantly increased in cells treated with the macroautophagy inhibitor 3-methyladenine (3MA) but substantially reduced in those treated with the macroautophagy inducer rapamycin. Conversely, the FK-derived PrP^{Sc} levels in cells treated by proteasome inhibitor had no effect. Furthermore, the decrease in FK-derived PrP^{Sc} levels was mediated, at least in part, by the phosphatidylinositol 3-kinase/MEK signaling pathway. By contrast, neither rapamycin nor 3MA had any apparently effect on PrP^{Sc} from either the 22L or the Chandler strain, indicating that the degradation of PrP^{Sc} in host cells might be prion strain-dependent. Collectively, our finding indicates that we might have to alter the therapeutic strategies by patients with Creutzfeldt-Jakob disease, and suggests the need for new therapeutic strategies, such as the use of autophagy-inducing compounds, in patients with GSS syndrome.

P-084: Differential effects of anti-prion treatments highlight differences in the pathogenesis of sporadic and familial forms of prion disease and identifies novel mode of action for pentosan polysulfate

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Early detection and disease modifying treatment of neurodegenerative disorders is essential for the preservation of nervous system function and patient quality of life. Familial forms of prion disease, associated with mutations in the prion protein gene, are arguably the most attractive candidates for timely prophylactic intervention once kindred's have been identified. However very few studies have considered whether the therapies that are effective against misfolded forms of the prion protein with a wildtype sequence (representing sporadic disease) are also effective against misfolded proteins with mutant prion protein sequences (representing familial disease).

In the current study we have infected RK13 cells expressing wildtype murine PrP^{C} or mutant PrP^{C} carrying a proline to leucine mutation at murine residue 101 (equivalent to P102L in human PrP) with the M1000 strain of mouse adapted human prions and investigated their susceptibility to curing when treated with congo red, chlorate, pentosan polysulfate (PPS) or cholesterol.

We found decreased levels of PrP^{Sc} in prion-infected cells expressing wildtype PrP^{C} following treatment with congo red, chlorate, PPS and cholesterol. Reduced PrP^{Sc} was confirmed by increased incubation period following *in vivo* bioassay of cell lysates. In contrast, the

levels of PrP^{Sc} present in prion-infected cells expressing mutant PrP^{C} was reduced following treatment with congo red and chlorate but unaffected by treatment with PPS and cholesterol.

Mutant and wildtype PrP^{C} were similarly expressed on the cell surface of RK13 cells and colocalised with markers of the endoplasmic reticulum. Mutant PrP^{C} was observed to have a slightly modified conformation around residue 111 which correlated with exposure of a cryptic binding site associated with glycosaminoglycan binding. However this did not affect the binding of PrP^{C} to glycosaminoglycans. Rather, we observed a decreased proportion of mutant PrP^{C} in lipid rafts and significantly increased levels of cell associated cholesterol in RK13 cells expressing mutant PrP^{C}. We observed a significant increase in cell associated cholesterol levels and a change in the localization of cholesterol following treatment of cells with cholesterol and PPS. We therefore conclude that PPS and cholesterol cure prion infected cells expressing wildtype PrP^{C} through affects on lipid raft integrity and that failure to cure prion infected cells expressing mutant forms of PrP^{C} reflects the presence of a non-lipid raft associated pool of PrP^{Sc}. This study highlights a difference in susceptibility of sporadic and familial disease to treatment and further offers an alternative mode of action for the anti-prion affects of PPS.

P-085: Unaltered prion pathogenesis in ER stress-prone mice

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Endoplasmic reticulum (ER) stress and the unfolded protein response have been implicated in the pathogenesis of acquired prion diseases. SIL1 is a nucleotide exchange factor for the ER chaperone BiP, which plays a crucial role in protein folding and UPR activation. Loss-of-function mutations in the SIL1 gene cause Marinesco-Sjögren syndrome (MSS), a rare genetic disease characterized by cerebellar ataxia with loss of Purkinje cells (PC). Mice carrying 2 disrupted Sil1 alleles (Sil1-/-) develop a MSS-like syndrome with UPR activation and PC degeneration. In contrast, loss of a single Sil1 allele does not cause disease, but enhances ER stress and exacerbates neurodegeneration in a mouse model of amyotrophic lateral sclerosis (Filézac de L'Etang A. et al, 2014). To investigate whether enhanced ER stress due to loss of SIL1 function exacerbated prion pathogenesis, we monitored the course of illness in RML-infected Sil1+/+ and Sil1+/- mice. We found no difference in time to onset of clinical signs and survival between the 2 groups of mice, indicating that reduced SIL1 activity does not significantly influence disease progression. Biochemical and histological analyses are in progress to assess the levels of ER stress markers and neuropathological changes in the inoculated mice.

P-088: Transmission of experimental CH1641-like scrapie to bovine PrP overexpression mice

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Introduction. Scrapie is a prion disease in sheep and goats. CH1641-lke scrapie is characterized by a lower molecular mass of unglycosylated form of abnormal prion protein (PrP^{Sc}) as compared to that of classical scrapie. It is worthy of attention because of the biochemical similarities of the abnormal prion protein $PrPPrP^{Sc}$ from CH1641-like and BSE affected sheep. We have reported that experimental CH1641-like scrapie is transmissible to bovine PrP overexpression (TgBoPrP) mice (Yokoyama et al. 2010). We report here the further details of CH1641-like scrapie transmission study and compare the

biological and biochemical properties to those of classical scrapie affected TgBoPrP mice.

Method. The details of sheep brain homogenates used in this study are described in our previous report as described above. TgBoPrP mice were intracerebrally inoculated with a 10% brain homogenate of each scrapie strain. The brains of mice were subjected to histopathological and biochemical analyses.

Results. $PrPPrP^{Sc}$ banding pattern of CH1641-like scrapie affected TgBoPrP mice was similar with that of classical scrapie affected ones. However, survival period was different between them. Mean survival time of CH1641-like scrapie affected TgBoPrP mice was 170 d at 3rd passage and it was significantly shorter than that of classical scrapie affected ones (439 days) at the same passage number. Transmission study using TgBoPrP mice demonstrates that CH1641-like scrapie is likely to be more virulent than classical scrapie in cattle.

P-091: Variant CJD: Lessons in public health

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Variant CJD was first reported in 1996. To date, there have been 177 cases reported in the UK and 52 cases worldwide in 11 different countries. Now, 20 y later, this fatal neurodegenerative disease remains a concern for public health, most notably regarding the potential for subclinical infection.

We have undertaken comprehensive transmission studies of vCJD cases. CNS tissue from a number of non-UK cases and UK cases selected from the early, mid and late points in the UK epidemic have been experimentally transmitted. Following first passage to wild type mice, we have established that both UK and non-UK cases show evidence of infection with the same vCJD strain of agent; consistent with our previously published studies, including a detailed analysis of the clinical, biochemical and pathological features of British and French vCJD cases. Secondary passage of selected cases are underway in order to fully assess strain characteristics and confirm that any minor differences are due to species barrier effects. Overall our results demonstrate that the vCJD strain has remained stable throughout the past 20 y in both UK and non-UK countries.

In a unique case study, we have now carried out experimental transmission with CNS tissue sampled from 2 Spanish cases of vCJD; a mother and son who resided in a BSE endemic area, and are thought to have ingested bovine brain. The resulting strain characteristics are similar to that of UK cases indicating that both individuals were infected by BSE and supporting the hypothesis of risk via ingestion of high titer bovine material.

Numbers of reported vCJD cases have continued to decline since 2000, indicating that control measures have been successful in limiting human exposure to the BSE agent. However, prevalence studies suggest that 1 in 2000 individuals in the UK carry disease-associated prion protein in peripheral tissues. A link between this protein deposition and vCJD infectivity has not been established, although we have shown that PrP deposition in the spleen of an asymptomatic individual is capable of transmitting infectivity in our mouse transmission studies.

We need to develop a better understanding of the risk of vCJD infection to both the individual and to the population and aim to address this by taking research and surveillance forward in a fully integrated manner to assess the risks of vCJD to public health.

P-092: Identification of new molecular alterations in Fatal Familial Insomnia

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Fatal familial insomnia (FFI) is an autosomal dominant prion disease caused by a D178N mutation in PRNP in combination with methionine at codon 129 in the mutated allele of the same gene. The present study analyzes pathological and molecular features in 7 FFI cases carrying the mutation D178N and M homozygous at the codon 129 of PRNP. Severe neuronal loss and marked astrocytic gliosis was observed in every case in the mediodorsal and anterior nuclei of the thalamus whereas the entorhinal cortex (EC) was variably affected. Spongiform degeneration was only observed in the EC. Synaptic and fine granular PrP^{Sc} immunoreactivity was found in the $_{EC}$ but not in thalamus. Microglia was barely increased in the mediodorsal thalamus, but mRNA expression of IL6, IL10RA, CSF3R and TLR7 was found in the thalamus in FFI. PrP^{C} levels were significantly decreased in the thalamus, $_{EC}$ and cerebellum in FFI compared with controls. However, increased expression of the non-glycosylated band of about 19 kDa was observed in the thalamus when using PrP antibodies mapping to the central region of the PrP comprising the α-helix domains H1 and H2. Decreased PrP mRNA levels were also observed in the thalamus and $_{EC}$. Altered PrP solubility was observed in FFI compared with controls; significantly reduced PrP levels in the cytoplasmic fraction and increased insoluble levels were found in FFI cases when compared with controls. Amyloid-like deposits were only seen in the $_{EC}$. RT-QuIC assay which mimics *in vitro* the conversion of PrPc to misfolded and amyloid PrP revealed that all the FFI samples of the entorhinal cortex were positive whereas the thalamus was positive only in 3 cases; the cerebellum was positive in 2 cases. All controls were negative. The expression of subunits of mitochondrial respiratory complexes and components of the protein synthesis machinery from the nucleolus to the ribosome was analyzed in the mediodorsal thalamus. NDUFB8 (complex I subunit), SDHB (complex II subunit), UQCRC2 (complex III subunit), COX2 (complex IV subunit) and ATP50 (complex V subunit) expression levels were reduced in FFI. Voltage-dependent anion channel and ATP5H were also reduced. In contrast, a marked increase in superoxide dismutase 2 was found in reactive astrocytes. The histone-binding chaperones nucleolin and nucleoplasmin 3, and histone H3 di-methylated K9 were markedly reduced together with a decrease in the expression of protein transcription elongation factor eEF1A in mediodorsal thalamus.

P-093: An autopsy case of MM1-type sporadic Creutzfeldt-Jakob disease with 1-month total disease duration presenting with early disease pathology

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Here, we report an autopsy case of MM1-type sporadic Creutzfeldt-Jakob disease (CJD) with 1-month total disease duration. The patient was a 62-year-old Japanese man with no family history of prion disease or known iatrogenic exposure to CJD. The initial symptom was abnormal behavior and he showed rapidly progressive neurologic signs, such as cognitive impairment, irritability, gait disturbance, and visual hallucination. Diffusion-weighted images (DWI) from magnetic resonance imaging (MRI) obtained 2 weeks after the onset of symptoms showed extensive hyperintense regions in the cerebral cortex and basal ganglia. Myoclonus was recognized 3 weeks after symptom onset. The patient died of acute respiratory failure due to aspiration pneumonia 4 weeks after the onset of symptoms. At the point of death, myoclonus was still present and the patient had not reached an akinetic mutism state. Because the patient was markedly restless and resistant to treatment until just before his death, we were not able to perform electroencephalography or lumbar puncture. The brain weighed 1,300 g and showed no apparent atrophy of the cerebrum, brainstem, cerebellum, and spinal cord. Extensive spongiform changes were observed in the cerebral cortex, basal ganglia, thalamus, and cerebellar cortex, but gliosis was generally mild or not observed in these lesions. Neuropil rarefaction and neuron loss were not apparent. Prion protein (PrP) immunostaining showed extensive diffuse synaptic-type PrP deposition in the cerebral, brainstem, cerebellar, and spinal gray matter. Analysis of the PrP gene revealed no mutation with Met homozygosity at codon 129. Western blot analysis of protease-resistant PrP indicated type 1 PrP.

This case revealed significant pathologic changes of the earliest stage of CJD. The earliest pathologic involvement was suspected to be spongiform change, because spongiform changes in neuropils without gliosis, neuropil rarefaction, or neuron loss were observed in the present cases, whereas no lesions showing gliosis, neuropil rarefaction, or neuron loss without spongiform change were observed. In addition, PrP deposition by immunohistochemistry can be detected before the spongiform change based on the finding that extensive PrP deposition was observed in the present case. MRI findings and the pathologic observations suggest that the hyperintensity observed on DWI is more likely to reflect spongiform change than gliosis, neuropil rarefaction, or neuron loss. Thus, the present case provides significant insight into the relation between clinical and pathological findings, and into the progression of CJD pathology.

P-097: Genetic Creutzfeldt-Jacob disease with V180I mutation in Korea

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Background: Creutzfeldt-Jacob disease (CJD) is a rare neurodegenerative disorder with rapidly progressive dementia, cerebellar ataxia and myoclonus. Genetic CJD accounts for 10-15% of all CJD cases. genetic CJD due to V180I is extremely rare and most of caes have come from Japan. We report a case of genetic CJD with mutation V180I in which clinical feature and MRI findings are different from previous cases.

Case: A 78-years-old woman was admitted due to rapidly progressive cognitive decline. Three months before admission, she complained of memory disturbance and was diagnosed as dementia. Thereafter, she also showed gait disturbance and communication was impossible. No

affected family member with dementia was found. On examination, she was bed-ridden and revealed very stiff extremities which made proper examination impossible. Diffusion weighted and FLAIR brain MRI demonstrated diffuse cortical high signal intensity, more on the left side, involving medial occipital lobes posterior to the parieto-occipital sulcus, but the cerebellum was spared. EEG showed mild slowing but no periodic sharp and wave complexes. CSF examination revealed positive 14-3-3 protein with elevated Tau (2863.0 pg/ml) and AB42 (801.5 pg/ml). Sequencing of PRNP demonstrated a V180I mutation with methionin homozygote at codon 129.

Conclusion: This is a second report of genetic CJD with V180I mutation in Korea. High signal intensities in MRI involving medial occipital lobes posterior to the parieto-occipital sulcus and rapidly progressive cognitive decline were unusual findings compared with previously reported cases with V180I mutation.

P-099: A therapeutic approach for Creutzfeldt-Jakob disease by DNAzyme-mediated knockdown of the prion protein

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Creutzfeldt-Jakob disease (CJD) is a fatal neurodegenerative disorder for which proteinaceous infectious particles (prions) are the single causative agent. Constant conversion of the endogenous cellular Prion Protein PrP^{c} to the pathogenic conformational isomer PrP^{Sc} is obligatory for the characteristic formation of insoluble, pathological protein aggregates. Therefore, it has been implied that reducing PrP^{c} expression might alleviate symptoms and increase survival rates. Despite several efforts, no PrP knockdown technique has yet been demonstrated that would be safe and effective in the treatment of human CJD patients.

The 10-23 DNAzyme is a catalytic nucleic acid capable of hydrolyzing RNA in a sequence-specific manner. We designed several DNAzymes targeting accessible stretches of the PrP mRNA. These DNAzymes completely cleave a molar excess of short RNA target sequences in a multiple-turnover mode. In addition, structured full-length RNA is efficiently cleaved during *in vitro* transcription in the presence of DNAzymes. Mouse N2a neuroblastoma cells transiently expressing human PrP show decreased PrP^{c} expression levels when treated with DNAzymes.

Our data show a feasible approach to reducing Prion Protein expression *in vivo*. Due to the specificity of the DNAzyme low or no off-target effects are expected. This opens up a new perspective not only for the treatment and management of Creutzfeldt-Jakob Disease but also that of protein aggregation disorders in general.

P-100: Differential association of amyloid-β with PrP^{Sc} pathology in each genetic prion disease

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Introduction. Although depositions of scrapie isoform of prion proteins (PrP^{Sc}) and amyloid β (Aβ) proteins were reported to be colocalized in the brains of patients with Gerstmann-Sträussler-Scheinker disease (GSS) and Creutzfeldt-Jakob disease (CJD), the

pathological relationship among PrP^{Sc}, Aβ and tau proteins in each genetic prion disease (gPrD) has not been clarified. Here we describe detailed immunohistochemical findings of those proteins, and assesse the pathological relationship in each gPrD.

Methods. We prepared paraffin-embedded autopsied brain sections from patients with gPrDs with mutation of P102L (GSS), P105L (GSS), E200K (CJD), or V180I (CJD), and compared those of sporadic CJD with type 1 PrP^{Sc} and methionine homozygosity (MM1). The serial brain sections of the frontal lobes were cut and examined immunohistochemically using anti-3F4, anti-4G8, anti-AT8, anti-Aβ40 and anti-Aβ42 antibodies.

Results. In hematoxylin and eosin staining, combination of fused large vacuoles and typical spongiform, typical spongiform, severe vacuolations, and rarely spongiform change were observed in brain sections of V180I, E200K, P105L, and P102L respectively. Plaque formation of PrP^{Sc} in the brain of patients with GSS with P102L and P105L mutations, and synaptic pattern of PrP^{Sc} deposition in sCJD of MM1, gCJD with V180I and E200K mutations were observed. Remarkably, about half of the PrP^{Sc} plaques were colocalized with Aβ42, but not with Aβ40, and several plaques were accompanied by dystrophic neurites with AT8 immunoreactivity in GSS with P105L. In GSS with P102L, PrP^{Sc} -Aβ42 colocalized plaques were barely observed, and neither tau pathology nor Aβ40 deposition was detected. In gCJD with V180I, Aβ42 immunoreactivity without Aβ40 was observed as plaque type deposition and no association with PrP^{Sc}, which was observed as very weak synaptic deposition. In gCJD with E200K, diffuse plaque depositions of Aβ42, which were often observed in aging brains, and clear synaptic deposition of PrP^{Sc} were observed. AT8 immunoreactivity was not detected in brains of any other gPrD than GSS with P105L.

Conclusion. The average disease durations were 58, 111.2, 25.0, and 12.8 in gPrDs with mutation of P102L, P105L, V180I, and E200K respectively. The PrP^{Sc} -Aβ42 colocalized plaques accompanied by tau pathology were observed predominantly in P105L mutation. Barely PrP^{Sc} -Aβ42 colocalized plaques without tau pathology were associated with P102L mutation. There was no association between PrP^{Sc} and Aβ deposition in synaptic types of PrP^{Sc} deposition in gPrDs. We speculate that the differences in pathological association of those proteins might cause an effect on the differentiation of their clinical features and pathological mechanisms.

P-102: Brain Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) and neuropathologic correlations in human prion diseases

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Introduction. Human prion diseases are invariably fatal rapidly progressive neurodegenerative illnesses. Definitive diagnosis is made only after neuropathologic examination, and clinical diagnosis is made based on clinical symptoms and the results of electroencephalograms, cerebrospinal fluid tests, and brain magnetic resonance imaging. Brain Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) is a metabolic neuroimaging modality that is commonly used in other neurodegenerative conditions such as Alzheimer's disease and frontotemporal

dementia. There is little data on brain FDG-PET scan results in cases of human prion disease and no data outside of case studies on neuropathologic correlations of brain FDG-PET scans.

Methods. A retrospective study was conducted that examined brain FDG-PET scans in human prion diseases. The following prion diseases were examined (13 total): 8 definite sporadic Creutzfeldt-Jakob disease (sCJD), 2 definite genetic CJD (gCJD, E200K-129M), 1 case of fatal familial insomnia (FFI), and 2 probable cases of sCJD. Brain FDG-PET scans were read by two different neuroradiologists and automated analyses were conducted using MIM Neuro software. Autopsies were conducted and subtype was delineated according to the Parchi et al. classification (Parchi P et al., 1999).

Results. Automated brain FDG-PET analyses demonstrated several similarities across cases. Hypometabolism was frequently demonstrated in precuneus (100%), posterior cingulate gyrus (92%), and cuneus (77%) regions. Surprisingly, we also detected hypermetabolism in the limbic and mesolimbic regions, including the insula (100%), medial temporal lobe (92%), amygdala (85%), and hippocampus (85%). When compared to diagnostic neuropathologic hallmarks of prion diseases, neuronal loss, astrocytosis, and spongiosis; hypometabolism was predictive of neuropathological changes in 81% of cortical regions compared to only 18% of subcortical regions. There were no significant differences between the patterns of PET scans performed in the first half of the illness versus the second half. In select cases, there were lower levels of reactive astrocytosis and spongiosis in areas of FDG-PET hypermetabolism.

Conclusions. Brain FDG-PET correlates well with neuropathologic changes in cortical areas, but not subcortical areas in human prion disease. Further studies are needed to examine if there are common brain FDG-PET findings across prion diseases and whether it can be used to differentiate distinct clinicopathological phenotypes and molecular subtypes of human prions.

P-103: Celia's encephalopathy: A new member of the group of protein misfolding-mediated neurodegenerative diseases

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We recently described a new very rare genetic neurodegenerative disease associated to a novel mutation, c.985C>T, in the BSCL2 gene. We have dubbed the disease (MIM 615924, Progressive Encephalopathy with or without Lipodystrophy) Celia´s encephalopathy, in memory of the index case. The gene product of BSCL2, seipin, is an integral membrane protein of the endoplasmic reticulum that plays an important role in the generation of lipid droplets and in adipogenesis. Most known mutations in the BSCL2 gene result in unstable versions of seipin, leading to a recessive, loss of function phenotype of generalized lipodystrophy. Alternatively, 2 specific missense mutations that interfere with its proper glycosylation, result in seipin variants that misfold and aggregate in aggresomes, which in turn results in dominant motor neuron diseases characterized by a neuromotor phenotype. In stark contrast, the c.985C>T mutation results in a severe neurodegenerative syndrome with a fatal outcome at age 6-8. To date, we have identified 6 cases of the disease, all in a small area in southeastern Spain. The pathological study of the index case showed widespread neurodegeneration, and, surprisingly, intranuclear inclusions positive for seipin. All in all, the pathology was very reminiscent of Huntington´

s disease. Genetic and biochemical studies showed that Celia´s mutation generates an alternative splicing site leading to skipping of one complete exon (exon 7), a change in the reading frame, and C-terminal truncation. The resulting seipin variant thus exhibits a highly aberrant C terminus. Sucrose density sedimentation studies show that while wild type seipin forms dodecamers, as previously described, Celia seipin forms very large, abnormal aggregates. Furthermore, proteomic analysis of preadipocytes of the index case suggest an ER stress response. So far there is no evidence that Celia seipin aggregates are able to induce misfolding of wt seipin; much to the contrary, we have found that when expressed in excess (10:1 ratio) with respect to Celia seipin, a situation that mimics the pattern of relative expression of both forms in heterozygous carriers of the mutation, wt seipin co-opts Celia seipin into heterologous multimers of the right size, thus neutralizing its tendency to form large aggregates. This might explain the puzzling complete lack of clinical manifestations in these individuals. Thus, one could say that normally folded wt seipin acts as a *reverse prionoid* under these circumstances.

P-104: AR-12 and its derivatives, a potential new therapeutic agent against prions

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Background: AR-12 is an FDA IND approved compound. It has previously been evaluated in a phase 1 clinical trial as an anti-cancer agent, but has also demonstrated host-directed, broad-spectrum clearance of bacteria and viruses. It is an orally available small molecule with robust human safety and is also known to cross the blood-brain barrier. Mechanistic studies suggest that AR-12 down regulates the host cell chaperone machinery, preventing the proper folding of viral proteins and efficient viral assembly. Additionally, AR-12 has been shown to downregulate GRP78, resulting in up-regulation of Atg13 and PERK, which induces autophagy and facilitates the clearance of intracellular viruses and/or unfolded proteins. The latter makes AR-12 an interesting target for treatment of neurodegenerative disorders. This is the first report on the effects of AR-12 in prion-infected cells. Previously, our lab has shown that autophagy stimulation by several chemical compounds can counteract prion infection *in vitro* and *in vivo*. In this study, we are investigating the role of AR-12 as a promising therapeutic agent that can promote clearance of prion infection.

Results: We tested AR-12 in various neuronal and non-neuronal cell lines infected with prions using different non-toxic drug concentrations, length of treatments and prion strains. AR-12 demonstrated a robust effect on decreasing the PrP^{Sc} level in prion-infected neuroblastoma cells (ScN2a). Upon three days of treatment with a single dose of 3 μM, PrP^{Sc} was almost undetectable in immunoblot analysis. This very fast anti-prion effect strongly suggests that the compound may have additive effects and also act on PrP^{Sc} clearance. In line with this, PrP^{Sc} decrease was accompanied by upregulation of autophagy markers like LC3-II. Using ScN2a cells with CRISPR/Cas9-based autophagy knock-out (Atg5 gene) proved that autophagy is involved in the mode of anti-prion action of AR-12. The anti-prion effect was validated in prion-infected CAD-5 neuronal cells and non-neuronal mouse embryonic fibroblasts (MEF). Interestingly, the effect was conserved in MEFs infected with 3 different prion strains (22L, RML and Me7). The AR-12 derivatives AR-13, -14, -15 and -16 were also evaluated. Interestingly, AR-14 was even effective at nanomolar concentrations. Therapeutic *in vivo* experiments in prion-infected mice and studies with models for other neurodegenerative disorders are planned.

Conclusion: This first study of AR-12 in prion-infected cells demonstrates that AR-12 is a promising new therapeutic agent for prion diseases and possibly protein misfolding disorders involving prion-Like mechanisms.

P-105: An autopsy-verified case of FTLD-TDP with upper motor neuron predominant motor neuron disease mimicking MM2-thalamic-type sporadic Creutzfeldt-Jakob disease

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The diagnosis of MM2-thalamic-type sporadic Creutzfeldt-Jakob disease (sCJD) is currently possible only through postmortem evaluation. To date, the clinical diagnosis of MM2-thalamic-type sCJD remains quite challenging because of the lack of laboratory or MRI characterization. Reportedly, the only supported diagnostic hallmark among live patients is decreased bilateral thalamic cerebral metabolism detected by FDG-PET or cerebral blood flow (CBF) detected by SPECT.

We report on a case of autopsy-verified FTLD-TDP with upper motor neuron predominant motor neuron disease mimicking MM2-thalamic-type sCJD. A 69-year-old woman presented with an 11-month history of progressive dementia, irritable psychiatric symptoms, and gait disturbance, without a family history. Neurological examination revealed severe dementia, frontal signs, and exaggerated bilateral tendon reflexes. Periodic sharp-wave complexes (PSWCs) were not observed on EEG. Brain diffusion MRI did not reveal abnormal changes. An eZIS analysis for ECD-SPECT revealed a bilateral decrease in thalamic CBF. PRNP gene analysis revealed methionine homozygosity at codon 129 without mutation. Cerebrospinal fluid (CSF) analysis showed normal levels of both 14-3-3 and t-tau protein. Conversely, PrP protein was slowly amplified in the CSF by RT-QUIC. Her symptoms deteriorated into akinetic mutism, and she died of sudden cardiac arrest in September 2014, one year after onset.

In this case, findings on SPECT supported a clinical diagnosis of MM2-thalamic-type sCJD; however, a postmortem assessment revealed that this was a case of FTLD-TDP type A. TDP-43 positive inclusion bodies were found in the frontotemporal cortices, amygdala, and the dentate gyrus of the hippocampus. PrP immunostaining did not reveal obvious PrP deposits in the CNS. Neuronal degeneration in the inferior olivary nuclei was not found. Moreover, Western blot analysis of protease K resistant PrP was performed with homogenized cryopreserved right frontal lobe and thalamic sections, which revealed no PrP positive band.

Decreased bilateral thalamic CBF detected by ECD-SPECT study may be diagnostically useful; however, it is not sufficiently specific to MM2-thalamic-type sCJD, and postmortem diagnosis of MM2-thalamic-type sCJD remains the gold standard for the diagnosis of this condition.

P-106: Temporal resolution of PrP^{Sc} transport, PrP^{Sc} accumulation, activation of glia and neuronal death in retinas from C57Bl/6 mice inoculated with RML scrapie: Relevance to biomarkers of prion disease progression

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Currently, there is a lack of pathologic landmarks to objectively evaluate the progression of prion disease *in vivo*. The goal of this work was to determine the temporal relationship between transport of misfolded prion protein to the retina from the brain, accumulation of PrP^{Sc} in the retina, the response of the surrounding tissue to PrP^{Sc} accumulation and resulting neuronal death in a mouse model of scrapie.

C57Bl/6 mice were inoculated intracranially with RML scrapie. Animals were euthanized and retinal samples collected at 30, 60, 90, 105, 120 d post inoculation (dpi) and at the onset of clinical signs of disease (153 dpi average). Retinal homogenates were prepared for RT-QuIC analysis and whole globes were fixed for standard immunohistochemical analysis. Antibodies against the prion protein (6H4), glial fibrillary acidic protein (GFAP), microglia (Iba-1) and activated microglia (CD68) were used to assess accumulation of PrP^{Sc} and the resulting response of retinal tissue. Loss of photoreceptors was used as a measure of neuronal death, and was quantified using nuclear counts on hematoxylin counterstained slides.

PrP^{Sc} seeding activity was first detected using RT-QuIC in all samples at 60 dpi, which was approximately 40% of the total incubation of 153 d. Accumulation of PrP^{Sc} with coincident activation of retinal glia was first detected at 90 dpi, which was approximately 60% of the total incubation period. Activation of microglia was first detected at 105 dpi (70% of the total incubation period), but significant neuronal death, measured by loss of photoreceptor cells, was not detectable until 120 dpi, which was approximately 80% of the total incubation period.

Our results demonstrate that by using the retina, we can resolve the temporal separation between several key events in the pathogenesis of prion disease. We have described a model with sufficient temporal resolution to study the relationships between transport, accumulation, resultant tissue activation and neuronal death. This model can be used to both study the mechanisms that underlie these events and evaluate the anti-prion activity of a variety of compounds *in vivo*.

P-108: Prospective surveillance data of human prion disease in the Chugoku and Shikoku regions of Japan

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Introduction. Genetic prion diseases in Japan have been reported to have a characteristic distribution of phenotypes that appears quite different from that of Western countries. Particularly in the Chugoku and Shikoku regions, which are rural areas with elderly populations in West Japan, there is a high frequency of genetic prion disease with PRNP mutation at codon V180I. The clinical features of V180I is significantly higher age of onset than that observed for sporadic Creutzfeldt-Jakob disease (sCJD).

Patients and Methods. From April 1999 to December 2015, 208 patients with sporadic CJD and 46 patients with genetic CJD were registered in the Chugoku and Shikoku regions,

areas that comprise a total of 8 prefectures. Thirty-four patients with genetic prion diseases (73.9%) had PRNP mutations at codon V180I. We analyzed the incidence rate of sCJD and V180I CJD in each prefecture in both these regions. To clarify the relationship between population aging and CJD, we also divided the surveillance period into 2 halves and compared the incidence rate of CJD between the 2 periods.

Results. The frequency of the V180I mutation in genetic CJD in these regions was significantly greater than that observed in the rest of Japan (about 40%). With respect to geographical analysis, the annual incidence rates of sCJD per million patients were clearly higher in the Shimane and Kochi prefectures than in the others. In contrast, the incidence rate in patients with the V180I mutation was significantly higher in the Ehime prefecture than in other prefectures. When assessing the first and second halves of the surveillance period, both sCJD and V180I CJD had a significantly higher incidence rate in the second half compared with that in the first half of the surveillance period. In addition, the age of onset in sCJD was significantly higher in the second half of the surveillance period compared with that in of the first half.

Discussion. The profiles of genetic prion diseases in the Chugoku and Shikoku regions were different from other regions in Japan, particularly in patients with V180I mutation.

It is important to consider that the rate of aging in these regions shows 24.6% growth from 2002 to 2015, but the geographical distribution and the growing incidence rate of CJD in these regions are unlikely to be fully explained only by an aging population. Other factors, such as the recognition of CJD, and MRI penetration rate may also have influenced the results.

P-110: Detection of disease-specific PrP and infectivity in the blood of mice with preclinical prion disease - Implications and applications for public health

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The accumulation of pathological isoforms of the prion protein, PrP, is a hallmark of variant Creutzfeldt-Jakob disease (vCJD), a fatal neurodegenerative disorder associated with exposure to bovine spongiform encephalopathy prions via the food chain. Recent studies indicate that although clinical cases of vCJD are rare, many more people are carriers of abnormal PrP isoforms, raising concern about the potential for perpetuation of infection via medical procedures, in particular transfusion of contaminated blood products. Accurate biochemical detection of infection is crucial to mitigate this risk and we have developed an assay capable of detecting abnormal PrP conformers present at the earliest stages of preclinical prion infection in experimental rodent models. This assay could be further developed to enable the screening of asymptomatic individuals (e.g., blood donors) for the protection of public health. Testing samples from preclinically infected mice using a combination of assays revealed that the ensemble of abnormal PrP conformers that accumulates throughout the incubation period of disease differ with respect to their infectivity. This raises the possibility of developing further biochemical tools to detect and characterize these species, thus shedding light on the mechanisms involved in prion infection and toxicity.

P-111: Iatrogenic Creutzfeldt-Jakob disease in human growth hormone recipients in the United Kingdom

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To date, 77 cases of accidental human-to-human transmission of Creutzfeldt-Jakob disease (CJD) have been reported in the United Kingdom (UK) in recipients of human growth hormone (hGH) derived from cadaveric pituitaries. Although hGH was withdrawn in 1985, cases of hGH-related iatrogenic CJD (hGH-iCJD) continue to occur. As part of a comprehensive analysis of hGH-iCJD cases, the prion protein (*PRNP*) genotype and the biochemical properties of the disease associated prion protein (PrP^{Sc}) were determined for a subset of UK hGH-iCJD subjects that were representative of the hGH-iCJD cohort as a whole. The 20 cases of hGH-iCJD were selected on the basis of consent for research and the availability of frozen tissue. Biochemical analysis of PrP^{Sc} in frozen brain tissue was performed by limited proteinase K digestion followed by Western blotting and the protease-resistant prion protein (PrP^{res}) was classified as either type 1 of type 2. The polymorphism at codon 129 of *PRNP* (MM, MV or VV) was determined by restriction fragment length polymorphism analysis from blood or from frozen brain tissue. Results were contrasted with comparable data from 108 UK sporadic CJD (sCJD) cases that occurred over a similar time period. Where possible, peripheral tissues from cases of hGH-iCJD were also investigated for the presence of PrP^{res}. In the hGH-iCJD patient group, we found a strong bias toward the MV and VV genotypes at polymorphic codon 129 of *PRNP*, combined with type 2 PrP^{res} (MV2 and VV2 subtypes). In contrast, and as expected, the MM1/MV1 subtype was the most frequently occurring subtype in the sCJD patient group. Cases of MM1 hGH-iCJD were rare, occurred late in the epidemic and displayed some unusual features. Despite the peripheral administration of hGH, very little evidence of PrP^{res} accumulation outside the brain was found. It has been proposed that human-to-human transmission of sCJD is a function of host *PRNP* codon 129 genotype and the strain of agent involved. Our results are consistent with UK hGH-iCJD resulting from exposure to prion infectivity from one or more sCJD patients, most probably of the MV2 or VV2 subtypes.

P-112: Assessing the disease-modifying role of TREM2 in a prion model of neurodegeneration

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Novel variants in TREM2 (triggering receptor expressed on myeloid cells-2) have been identified as risk factors for neurodegenerative disease including Alzheimer. TREM2 is a cell surface receptor on microglia that regulates homeostatic and immunomodulatory functions including phagocytosis of apoptotic debris and the resolution of damage-associated inflammation. Thus TREM2 has been identified as an important molecule for understanding the role of microglia in the resolution of the disease process. However, it is currently unclear if TREM2 has a causative role in neurodegenerative disease and recent studies using APP-overexpressing models of amyloidosis have provided inconclusive results.

Here we utilised a prion disease model to assess the role of TREM2 in the progression of a fatal neurodegenerative disease. Prion diseases are characterized by the accumulation of prion protein (PrP). The precise time course of disease induced phenotypes and clinical endpoints of neurodegeneration are extremely well characterized in prion models thus providing a robust platform to determine if TREM2 has a

disease-modifying effect on the neurodegenerative process.

TREM2 knockout and wildtype littermates were challenged intracerebrally with ME7 at 9-12 weeks of age. Animals were clinically scored throughout the disease course for neurodegenerative phenotypes (such as ataxia, piloerection). At the terminal stage of disease, brain sections were examined for disease pathology (vacuolation, PrP deposition and gliosis).

Incubation period of disease was comparable in wild type and TREM2 knockout animals. Histological examination revealed similar vacuolation profiles and PrP deposition. However, semi-quantitative analysis of microglial morphology suggested less activation in the absence of TREM2.

Our results identified that loss of TREM2 has no impact on the clinical stages of disease, end-stage vacuolation or PrP deposition but suggest potential alteration of microglial phenotype which may impact on preclinical disease process.

P-113: Two International Ring-trials demonstrate that CSF RT-QuIC is a robust and reliable test for diagnosing sporadic CJD

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Sporadic Creutzfeldt-Jakob disease (sCJD) is a fatal neurodegenerative disease belonging to the family of transmissible spongiform encephalopathies. It is clinically characterized by a rapidly progressing dementia alongside other cognitive and motor impairments. Diagnosis of pre-mortem sCJD is currently based on clinical features, the results of EEG, MRI and a positive 14-3-3 in the cerebrospinal fluid (CSF). However, none of these tests are specific for sCJD and all are surrogate markers. A major advance in the pre-mortem diagnosis of sCJD is the use of CSF real-time quaking induced conversion assay (RT-QuIC) analysis. This test exploits the ability of PrP^{Sc} to convert native PrP into PrP^{Sc} and is therefore specific for prion diseases. Using CSF RT-QuIC has been shown to have a high degree of sensitivity (85-87%) and specificity (99-100%) for the diagnosis of sCJD. However before being accepted as part of the diagnostic criteria for sCJD, RT-QuIC needs to be shown to be a robust, reproducible and easily standardised technique.

A JPND-funded consortium of international laboratories was established in 2012 to optimise, standardise and harmonise RT-QuIC analysis (CSFQuIC). As part of this project 2 separate international ring-trials were carried out over 2 y which involved 11 different laboratories, using a variety of instrumentation and recombinant PrP substrates. A total of 25 CSF samples were analyzed and the results showed near-complete concordance between laboratories, despite the variety of instrumentation and substrates used. This data strongly supports the inclusion of RT-QuIC in the clinical investigation of patients with suspected sCJD.

Additional authors of this work can be identified at: **http://tinyurl.com/hsrhbmr**

P-114: Enhanced Creutzfeldt-Jakob disease surveillance in the older population in the UK: Biochemical analysis for PrP^{Sc}

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Human prion diseases are invariably fatal neurodegenerative diseases associated with the conversion of a host protein (PrP^{c}) to a pathogenic, misfolded form (PrP^{Sc}). They occur as

idiopathic, genetic and acquired forms. Sporadic Creutzfeldt-Jakob disease (sCJD) is the most common, with a reported annual mortality rate of 1-2 individuals per million population per annum. Most sCJD cases are reported in the 65-79 y age group and thereafter the reported numbers of cases rapidly decline. Variant CJD (vCJD) is an acquired form linked to exposure to the bovine spongiform encephalopathy agent. In contrast to sCJD, vCJD generally affects younger individuals with a median age of onset of 27 y. All definite clinical cases of vCJD to date have occurred in individuals homozygous for methionine at *PRNP* codon 129. There is a concern that vCJD might occur in individuals with other codon 129 genotypes, but after longer incubation periods. Consequently, these patients might be older, and there may be age and genotype related variation in clinical and pathological features of the resultant disease. There is a further concern that all forms of human prion disease, including sCJD and variably protease sensitive prionopathy (VPSPr) might be under-ascertained in the older age groups due to the prevalence of dementia in older people and the low rate of referral to neurological services and subsequent autopsy investigation. To address these concerns we are conducting a feasibility study of methods to identify unrecognised prionopathy in individuals who are 65 or more years of age. As part of this study we are performing in depth testing of brain tissue donations to the MRC Edinburgh Brain Bank for evidence of prion disease. In a second complimentary approach we are also investigating patients presenting to local neurology and psychogeriatric services with dementia with atypical features and who subsequently donate to the MRC Edinburgh Brain Bank. Frozen samples from 6 defined brain regions from each donated brain are analyzed using a range of approaches that are designed to maximise detection of the differing forms of PrP^{Sc} associated with vCJD, sCJD and VPSPr. The methods used are Western blotting for protease resistant PrP^{Sc} (WB), sodium phosphotungstate (NaPTA) precipitation of PrP^{Sc} prior to Western blotting (NaPTA-WB), conformation dependent immunoassay (CDI) and the *in vitro* amplification techniques protein misfolding cyclic amplification (PMCA), and real time quaking induced conversion (RT-QuIC). The experience of the first year of this study will be presented.

P-115: Accuracy of Creutzfeldt-Jakob disease diagnosis using RT-QuIC testing of olfactory mucosa and cerebrospinal fluid samples

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Objective. We previously detected prion seeding activity in olfactory mucosa (OM) of Creutzfeldt-Jakob disease (CJD) patients using nasal brushings coupled with real time quaking induced conversion (RT-QuIC) with 100% specificity and >97% sensitivity. OM samples were collected using disposable cyto-brushes that might cause mild discomfort or recovery of blood cells. To further evaluate and improve this approach to CJD diagnosis, we tested a softer, less abrasive flocked swab. Moreover, we assessed the diagnostic outcome of combined RT-QuIC analyses of OM and cerebrospinal fluid (CSF).

Methods. We collected OM and CSF samples from 61 possible, probable or definite CJD, 8 genetic prion disease, as well as50 OM and 54 CSF negative control samples and analyzed them by RT-QuIC.

Results. OM samples collected with swab contained less blood contamination than cyto-brush samples. Single cyto-brushings (n = 48) or swabbings (n = 95) of sCJD patients gave diagnostic sensitivities of 90-93%. Cumulative results from 3 successive nasal samplings improved sensitivity to 97%. CSF testing of the same patient group was 95% sensitive. Collectively, all sCJD patients (n = 61) were RT-QuIC-positive using either OM, CSF, or both, giving an overall RT-QuIC diagnostic sensitivity to 100%. All non-CJD controls were negative, giving 100% specificity. For patients with genetic prion disorders, combined OM and CSF testing was 75% sensitive.

Interpretation. OM swab sampling alone allowed gentler, yet highly accurate, sCJD testing. However, the ability to consecutive testing of both CSF and OM samples increased the diagnostic accuracy to 100% for the sCJD patients tested to date.

Supported in part by Alliance Biosecure Foundation and University of Verona OLFASSAY

P-116: A novel approach combining 3D human cell culture and 3D microscopy to assess prion infectivity

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New approaches based on *in vitro* organoids to model human diseases are an intense area of research. The complexity of these new 3-dimensional (3D) models may range from the simplest, with cell lines-derived spheroids, to the most elaborate ones, with organoids derived from Induced Pluripotent Stem Cells (iPSCs). Recently was described a protocol designed to obtain human neuroectodermic 3D organoids termed 'Mini-Brains' (Lancaster et al., Nature 2013), allowing more than 1 y long-term culture with the potential to model later events such as neuronal maturation and survival. Potentially, 3D human cell culture could bridge the gap between traditional 2D cell culture and animal models because it mimics an *in vivo*-like microenvironment in an adaptable experimental platform.

The goal of our study is to define the conditions of a 3D human cell culture model susceptible to infection by prions and adapted for drug discovery in High-Content Screening (HCS). The complex structures obtained, with variable tissue organization can be studied in HCS in automated 3D imaging. Through this work, we are currently developing several original approaches to overcome the issues of this strategy by combining a large panel of techniques: 3D human cell culture, optical clearing, epitope unmasking techniques, immunofluorescence staining with multiple rounds of antibody probing-striping and the development of a 3D microscopy exploration system. We first tested this approach on 2 types of 3D models: spheroids derived from a human neuroblastoma cell line (SH-SY5Y), and neuroectodermic organoids derived from human iPSCs (Mini-Brains). These two models were contaminated with clarified brain homogenates from a sporadic Creutzfeldt-Jakob disease patient: here we present our preliminary results from this study.

P-117: Iatrogenic CJD after human GH treatment in France: Effect of sex, dose and genetics on the susceptibility of a possible infection by a V2 sCJD strain

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Background. Iatrogenic Creutzfeldt-Jakob disease results mainly, in France, from the administration of human cadaver-sourced growth hormone (hGH-CJD). In 2016, 239 cases were reported worldwide principally in the United States, United Kingdom and France where 119 cases have been identified so far. All of the French cases were treated between December 1983 and July 1985. The hGH-CJD series collected in France provides a unique opportunity to better understand how prion transmission occurs in humans.We studied major features within treatment protocol and host characteristics associated to the occurrence of hGH-CJD and those who influence the clinico-pathological phenotype.

Methods. The hGH treated patients who have not developed CJD so far (non hGH-CJD) were compared to hGH-CJD. Among the 119 hGH-CJDs, we studied clinical symptoms, genotype at codon 129 of PRNP, neuropathological data and biochemical properties of PrPsc.

Results. In the 1443 treated patients group and, surprisingly, even more in the hGH-CJD group, the male-to-female sex ratio was higher than 1. The hGH-CJD received a significantly higher number of hormone doses during a longer period of time compared to non hGH-CJD. Patients who were methionine homozygous at codon 129 were over-represented in hGH-CJD compared to the general population. Incubation period and disease's duration were significantly different between the 3 genotypes at codon 129. We identified hormone batches that were at risk for hGH-CJD occurrence. The Odd ratio varies according to the genotype. Patients with hGH-CJD had a fairly stereotypical clinical presentation marked by an inaugural cerebellar syndrome. Differences in neuropathological aspects were identified; all Met/Met and half of Met/Val cases showed florid plaques in cortex and cerebellum that were not observed in Val/Val cases. Lesion profiles in Met/Met and Met/Val groups were compared with those obtained in sporadic CJD. Distribution of lesions for both Met/Met and Met/Val resembled to that of sporadic Val/Val 2A. Among Met-Met, PrPres type 1 was the most common type while in Met/Val group type 2 was dominant.

Discussion. Susceptibility seems to be governed by sex, codon 129 and treatment protocol. Clinico-pathological phenotype is modulating by codon 129 (duration, florid plaques or not)/ Similarities in the clinical manifestations and lesion profile between Met/Met and Met/Val hGH-CJD and sporadic Val-Val 2A subtype suggest that, in addition to the route of contamination, an effect of the strain that could be a plaques-inducing prion agent that targets the cerebellum (such as the V2 sCJD one) influences the phenotype.

P-119: Prion protein interactome: Identifying novel targets in rapidly progressive Alzheimer's disease

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Rapidly progressive Alzheimer's disease (rpAD) is a variant of Alzheimer's disease (AD) with an aggressive course, exhibits distinct clinical and neuropathological features. Patients with rpAD show decline of more than 5 points of Mini Mental State Examination per year. Cerebrospinal fluid (CSF) biomarker profile of rpAD cases is similar to that of AD cases except occasional appearance of 14-3-3 and a higher TAU/A Beta1-42. The rapid decline of cognition and appearance of 14-3-3 in CSF suggest similar mechanisms to that of prionopathies and demonstrate a missing link between rapid progressive dementia and Prion protein (PrP).

Here, we investigate a possible correlation between PrP differential isoforms, with immunoblotting; and PrP interacting proteins in rpAD, in AD and in non-dementia controls. Prion protein along with its interacting proteins was affinity purified using magnetic Dynabeads protein G, in-solution digested, and identified by Q-TOF MS/MS analysis. Experiments were carried using post mortem frontal cortex samples from AD cases (age, M = 76.8, SD = 4.3 years), rpAD cases (age, M = 76.5, SD = 5.3 years) and non-demented controls (age, M = 77.5, SD = 5.9 years).

Interestingly, our data demonstrated significant 1.2 folds ($p<0.05$) decrease in diglycosylated PrP isoforms specifically in rpAD pateints in comparison with AD patients. However, from ESI/MS analysis of PrP specific interactome, 11 proteins appeared to interact with PrP. Out of 11, 2 proteins i) Histone H2B type 1-B and ii) Zinc α-2 protein were specifically bound with PrP isoforms from rpAD cases. Furthermore, 2 proteins i) Synaptojanin-1 and ii) synaptopodin showed no potential interaction with PrP isoform from rpAD cases and appeared to bind with PrP in only non-demented healthy controls. Interestingly, one protein Myelin P2 protein was characteristically isolated as binding partner with PrP isoform in AD samples only and demonstrating a possible link with slow progression of AD cases. Whereas, 2 interacting proteins i) peroxiredoxin-1 and ii) Myelin Proteolipid protein were commonly found in AD and rpAD cases with higher expression in AD. Lysozyme-C was found common in AD and non-demented healthy controls.

In conclusion, our data highlights the dysregulation of post translational modifications of PrP, leading to decrease diglycosylated Prp isoforms in rpAD, in association with the altered PrP interactome this could suggest PrP linked deregulations of Myelin metabolism, variations in synaptic transport and higher degree of oxidative stress going on in case of rapidly progressive Alzheimer's Disease.

Keywords: Co-immunoprecipitation, prion, rapidly progressive-AD

(Supported by Helmholtz-Alberta-Initiative - Neurodegenerative-Diseases-Research (HAI-NDR); Alberta-innovates-Biosolutions, and DZNE-Goettingen)

P-120: Assessment of doxycycline treatment on prion deposition in the olfactory epithelium of patients with Fatal Familial Insomnia: Possible mirroring of the CNS alterations

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Fatal Familial Insomnia (FFI) is an autosomal dominant inherited prion disease due to a point mutation at position 178 of the prion protein gene (PRNP) resulting in aspartic acid to asparagine substitution (D178N) in coupling phase with methionine at position 129. The overall amount of protease resistant prion protein (PrPres) in FFI is 5-10 times lower than that found in classical forms of sporadic CJD and prevalently affects the thalamus. Recent data showed that prions can be found in the olfactory mucosa (OM) of patients with CJD (Zanusso et al. 2014). The aim of the present study was to verify whether PrPres is also present in the OM of FFI patients and, if this were the case, whether this marker can be used to evaluate the response to pharmacological treatment. First, we optimized the protein misfolding cyclic amplification (PMCA) on samples of frontal cortex from FFI patients. The optimized method allowed to detect a PrPres signal till 10-12-fold dilution of brain homogenate. We then analyzed the OM from a symptomatic FFI patient and found a PrPres signal with a biochemical profile similar to that found in FFI brain. Quantitative PMCA showed that the PrPres concentration in the OM was approximately 1.4 × 10-18 grams/10 microliters. We are currently extending the study to members of a large Italian kindred of FFI involved in a clinical trial aimed to assess the effect of doxycycline (administered at preclinical stage) on disease progression. OM samples, collected at both preclinical and clinical stage of doxycycline-treated and -untreated individuals, will be analyzed to assess whether PrPres can be detected at a pre-symptomatic stage and if the treatment reduces the amount of PrPres or alters its biochemical properties. Since OM belongs to the CNS, the analysis of OM-PrPres might mirror the modifications occurring in the CNS, thus providing important information about the real effect of the doxycycline on prion propagation.

P-121: Unusually young prion disease cases in the United States, 1979-2014

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Introduction. Creutzfeldt-Jakob disease (CJD) occurs with a frequency of about 1.0-1.5 cases per million population per year in the United States. Most cases occur sporadically in older adults; the disease is rare in persons <30 y of age. We reviewed information for young

patients with a prion disease diagnosis who died in the United States during 1979-2014.

Methods. Prion disease decedents aged <30 y were identified from the US national multiple cause-of-death data for 1979-2014 and the National Prion Disease Pathology Surveillance Center (NPDPSC) database, which contains results of laboratory testing performed at the center. Whenever possible, medical records and neuropathological and genetic data were reviewed.

Results. During the 36-year period, a total of 23 decedents aged <30 y were identified with a prion disease diagnosis in the United States; 22 decedents could be further classified by prion disease type. Seven of the 22 decedents (31.8%) had a sporadic prion disease for an average annual incidence of 1.6 per billion. These 7 decedents included 6 with sporadic CJD and 1, the youngest decedent at 16 y old, with sporadic fatal insomnia. Seven decedents (31.8%) had iatrogenic CJD, 6 (27.3%) had familial CJD, and 2 decedents (9.1%), infected overseas, had variant CJD (vCJD). The 1 decedent with a prion disease of unknown type died in the early 1980s, making a vCJD diagnosis unlikely. The 7 iatrogenic cases, 4 associated with human growth hormone (hGH) and 3 associated with dura mater grafts, included the index cases of international iatrogenic CJD outbreaks linked to either pituitary-derived hGH or Lyodura brand dura mater grafts. Four of the familial cases had Gerstmann-Sträussler-Scheinker syndrome, 1 had fatal familial insomnia, and 1 had a G54S mutation. For young sporadic CJD decedents with genetic testing results available, 2 were methionine homozygous at codon 129, 2 were valine homozygous, and 1 was heterozygous.

Conclusion. In contrast to prion disease cases among older adults, only about one third of the unusually young cases were sporadic; over 40% had an exogenous source of infection. Given the rarity of young prion disease cases and the subset that have served as important initial signals for controllable outbreaks, the continued identification and full workup of cases aged <30 y is recommended.

P-123: Rapid testing for Creutzfeldt-Jakob disease in donors of human tissues

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Introduction. At least 6 iatrogenic transmissions of Creutzfeldt-Jakob disease (CJD) attributed to corneal transplantation were confirmed or suspected worldwide. Over 40,000 corneas are transplanted yearly in the US. In many countries, including the US, eye banks screen donors by history to identify risk factors and test for several communicable diseases. Medical review should exclude demented donors and others at increased CJD risk. But cadaveric donors of eyes and other tissues are not tested for CJD for several reasons: no marketed human CJD screening test, low prevalence of CJD and low overall autopsy rate. We believe that some of these logistical difficulties can be addressed.

Study Design. The optic nerves and frontal lobes of persons with CJD generally contain both infectivity and the abnormal prion protein (PrPTSE) that accumulates in transmissible spongiform encephalopathies (TSEs). We hypothesized that the small amount of tissue accessible after enucleation using a disposable biopsy punch and plunger that pass through a retro-orbital trocar hole into the brain should be sufficient to test for PrPTSE. Because we had no access to autopsies, we attempted instead to demonstrate that the volume of brain obtained with a 4-mm punch biopsy provided sufficient material to detect PrPTSE consistently. As a commercial test, we choose the IDEXX HerdChek BSE-Scrapie Ag Kit, a sensitive veterinary test detecting PrPTSE in brains of ruminants with TSEs.

Results. We obtained 3 separate tissue samples from 6 confirmed CJD-infected human brains stored at -80C, prepared 10% w/v brain

homogenates from each of the 18 specimens, and serially diluted them in half-log increments using 10% normal human brain homogenate as diluent. Samples were tested by IDEXX. We assayed control samples (10% w/v brain homogenates) from 28 individuals with several non-TSE neurodegenerative diseases and 10 normals.

We found no reactive results with any control brain suspension (100% specificity). All 18 infected specimens were positive at 1% and 0.3% brain dilutions. (One sample of a triplicate gave a low positive result, while the other 2 gave high positive results). IDEXX was at least 30-fold more sensitive than Western blot in detecting PrPTSE in diluted brain suspensions.

Conclusions. A postmortem rapid sensitive screening test for CJD is feasible without full autopsy and might enhance the safety of transplanted tissues. While results of this study suggest proof of principle, we do not endorse routine off-label use of any commercial veterinary test with human tissues.

P-124: Rational design and optimization of drug leads targeting prion-like misfolding and aggregation of SOD1 enzyme in Amyotrophic Lateral Sclerosis

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Misfolding of superoxide dismutase 1 (SOD1) enzyme due to multiple factors can result in formation of neurotoxic oligomers in Amyotrophic Lateral Sclerosis (ALS). Currently, no effective treatment of ALS is available. Several drug-like molecules were screened in silico to stabilize SOD1 dimer interface. Among these is 5-fluorouridine (5-FUrd) that binds near Trp32 residue of SOD1, not at the dimeric interface as originally proposed. It has been demonstrated that 5-FUrd and other small molecules binding at Trp32 can significantly inhibit transmission of SOD1 misfolding. It was previously shown that Trp32 is important for templated misfolding of SOD1 in the prion-like propagation of pathology in ALS [Grad et al., PNAS 108: 16398-403 (2011)]. Inhibition of SOD1 misfolding is one of the most promising strategies in ALS therapy.

The goal of our study is rational design of new drug leads targeting the Trp32 site of SOD1 for ALS therapy. Based on analysis of physico-chemical descriptors of the experimentally observable binding mode of 5-FUrd, new compounds were rationally designed. Their binding energies were calculated with the MM-GBSA method. All these compounds bind to the Trp32 site with higher affinity compared to 5-FUrd. The higher binding energies are attributed to additional favorable interactions emerged from substitution/introduction of new functional groups. Stability of new compounds and 5-FUrd in the binding site were analyzed through MD simulations. Residence time of 5-FUrd estimated based on the transition sate theory is approximately 9 ns, in agreement with the experimental data.

Further, the pharmacophore model was built by considering the molecular recognition features of 5-FUrd at the Trp32 site. The model was validated with the set of compounds including 5-FUrd. The screening of a large library of lead-like compounds (600,000 compounds) at the Trp32 site of SOD1 was performed. A few compounds with higher binding energy compared to 5-FUrd were identified.

Positive control docking experiments suggest that structural water a play major role in defining binding to the Trp32 site. To accurately account for solvation, we developed a new approach which couples the standard modeling tools (docking, virtual screening, and free energy calculations) with the statistical-mechanical, 3D-RISM-KH molecular theory of solvation. Placement of structural water with

the new approach significantly improves accuracy of binding modes prediction. This development will be essential for identification of top-ranked compounds from virtual screening of large library of compounds and de novo design of new compounds.

P-125: Novel detection technique of early Alzheimer's disease from blood using fluorescence spectral microscopy

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Neurodegenerative disorders are devastating diseases that include Alzheimer, Parkinson, and prion diseases. Evidence indicates that misfolding of cellular proteins, resulting in impaired clearance and accumulation, plays a central role in the pathogenesis. Alzheimer disease (AD) is pathologically characterized by Aβ-containing senile plaques, neurofibrillary tangles, and synapse loss in the brain. Toxic Aβ peptides aggregate into higher molecular weight assemblies and accumulate not only in the extracellular space, but also in the walls of blood vessels in the brain, increasing their permeability, and promoting immune cell migration and activation. Given the prominent role of the immune system, cellular elements in the blood could have opportunity to contact pathological brain materials, and therefore to act as "sentinels," with a "memory" of their experience after trafficking through the brain and its microvasculature. Despite considerable effort, there is neither a cure for AD, nor even a non-invasive clinical test to reliably establish the diagnosis with certainty. Thus, development of an inexpensive, non-invasive, rapid test for early AD detection is of paramount importance as the developed world braces for this inevitable epidemic. We developed a novel method for early diagnosis from blood. Using spectral imaging techniques with conformationally-selective fluorescent amyloid probes, we imaged fluorescence from circulating AD transgenic mouse (5xFAD) and human (AD, MCI and controls) blood leukocytes and RBCs. Cell type-specific amyloid accumulation patterns were identified by using our custom analysis method, and the results were validated against the diagnosis of clinical probable AD by NIA-AA criteria. A time course study of the 5xFAD mouse revealed that leukocyte Aβ accumulation was detectable as early as 2 months of age, paralleling the earliest signs of brain Aβ deposition. Moreover, peripheral Aβ signals quantitatively mirror the Aβ plaque load in the brain. In human blood samples, our spectral image analysis clearly distinguished between control, MCI and AD in both leukocytes ($p < 0.001$, MCI vs. control; n.s., AD vs. control) and RBCs ($p < 0.0001$, MCI vs. control; $p < 0.03$, AD vs. control). These observations suggest that our method is capable of detecting early AD pathology in circulating human blood cells, including RBCs, mirroring the "Aβ load" in the brain. Importantly, our method is able to detect pathological conformers of Aβ in human and transgenic mouse blood cells, and thus, may constitute a reliable and inexpensive test for detection of early AD and AD-related MCI.

P-126: Geographic risk of variant Creutzfeldt-Jakob disease: A risk ranking model to evaluate options for blood donor deferral policies in the US

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Background. To mitigate the risk of transfusion-transmitted variant Creutzfeldt-Jakob

disease (TTvCJD), the FDA has, since 1999, recommended deferring certain blood donors in the US who traveled to Western European countries and may have been exposed in excess of a period of time to the agent of bovine spongiform encephalopathy (BSE)-the agent causing human vCJD. Those recommendations warrant re-evaluation because the annual reported numbers of BSE and vCJD cases have abated markedly.

Study Design and Methods. We used an algorithm to rank national vCJD risk based on a few key factors. The risk contributed by each country was calculated based on its vCJD case rate and the person-years of exposure of US blood donors in the country. We used the reported vCJD case rate for a country, when available, or imputed vCJD case rates based on reported BSE cases and UK exported beef to assign a probable vCJD risk. We estimated resulting risk reduction and donor loss should the criteria for donor deferrals be narrowed. We further estimated the additional effect of leukocyte reduction (LR) of red blood cells (RBCs) in reducing TTvCJD risk.

Results. The UK, Ireland, and France have the greatest vCJD risk, contributing approximately 95% of total risk worldwide. The model estimated that deferring US donors who spent extended periods of time in these 3 countries, combined with voluntarily implemented LR (currently 95%), would reduce vCJD risk by 89.3%, a reduction similar to that achieved under current policy (89.8%) but with reentry of about 100,000 donors.

Conclusions. Our analysis suggests that a geographic vCJD deferral option focusing on 3 highest-risk countries would achieve a level of blood safety similar to that of the current deferral policy.

P-127: Early response of Cofilin1 pathway in Creutzfeldt Jakob disease

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Growing evidence suggests that synaptic failure, rather than the actual death of neurons, is the fundamental cause of neurological dysfunction in prion maladies. Creutzfeldt Jakob disease is the most common human prion disease caused by misfolding of the cellular prion protein (PrPc). Although accumulation of PrP^{Sc} has been associated with synaptic pathology in prion diseases, but the mechanism by which it leads to synaptic abnormalities is unknown. Cofilin1 mediated actin dynamics are required for synaptic plasticity.

So accordingly we sought to explore molecular changes in Cofilin1 pathway. Interaction of PrPc with cofilin1/Cofilin1-phospho (Ser3) was investigated in primary cortical neuronal cultures of PrPc wild type and knockout mice (PrP-/-) by Co-immunoprecipitation followed by confirmation with confocal laser scanning and co-sedimentaion assay. To Explore mechanisms regulating Cofilin1 activity, we investigated expressional changes in Cofilin1 and its up-stream regulators LIMK1-SSH1-ROCK2-APP, in Cerebellum and frontal cortex of 2 most frequent subtypes of human sCJD; MM1 and VV2 and in age matched healthy controls as well as in tg340 mice expressing about 4-fold of human PrP-M129 and PrP-

V129; at preclinical and Clinical stages of the disease.

Interestingly, we found a region and subtype specific regulatory response of Cofilin1 activity in sCJD. We observed an increase in Cofilin1 phosphorylation in Cerebellum of both subtypes and in frontal cortex of MM1 subtype of human sCJD as compared to healthy controls, suggesting an inactivation of Cofilin1 in diseased brain. There was a significant decrease in the levels of total cofilin1 in frontal cortex of MM1 and in cerebellum of VV2 subtype. In mice model Cofilin1 expression was significantly downregulated at pre-symptomatic and at early symptomatic stage of the disease in the frontal cortex; in contrast, upregulated in the Cerebellum. Further P-Cofilin (Ser3) showed significant upregulation in the frontal cortex and downregulation in cerebellum at different disease stages. Furthermore we found an altered/disrupted expressional pattern of upstream regulators of cofilin pathway LIMK1-SSH1-ROCK2-APP in sCJD MM1-Mice and in human MM1 and VV2 frontal cortex and cerebellum.

In conclusion, our results highlight molecular mechanisms leading to synaptic failure in sCJD with early regulatory response of cofilin1. Dysregulation of Cofilin1 mediated actin remodeling may be a major cause of neuronal dysfunction and degeneration in sCJD and possibly in other neurodegenerative maladies.

Keywords cofilin pathway, prion protein, transgenic mice, sporadic creutzfeldt Jakob disease, synaptic dysfunction.

Supported by Helmholtz-Alberta Initiative - Neurodegenerative Diseases Research (HAI-NDR); Alberta innovates Bio solutions, and DZNE Goettingen.

P-129: Epidemiologic features of human prion diseases in Japan: A prospective 15-year surveillance study

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A nationwide registration system for human prion diseases (hPrDs) in Japan, coordinated by the Creutzfeldt-Jakob Disease (CJD) Surveillance Committee, has been operating since 1999. We aimed to reveal the epidemiologic features of hPrDs in Japan by analyzing this 15-year database.

Information about patients suspected to have hPrDs was obtained from 3 sources: (1) the government's Intractable Disease Treatment Research Program, (2) the government's Infectious Diseases Control Law, and (3) requests from physicians to the CJD Surveillance Committee for genetic or cerebrospinal fluid analysis through Tohoku University and Nagasaki University, respectively. We analyzed data from all patients suspected of having hPrDs registered by the CJD Surveillance Committee between April 1999 and September 2015. All referrals were assessed using the World Health Organization case definition for hPrDs.

During the surveillance period, 5275 suspected patients were identified, and 2596

patients were confirmed as having hPrDs, including 1110 males (43%) and 1486 females (57%). Initially, patient numbers increased annually, peaking at 220–230 patients in recent years.

The annual incidence of hPrDs has tended to increase since 1999, with 1.8 patients per 1 million people in 2013 (= the latest year for which complete data was available due to a lag between disease onset and registration). Classified into younger (40–69 years) or older patients (70 year or more), the rate of hPrDs in younger patients is generally stable, but the incidence in older patients markedly increased during 1999–2013.

The hPrD subtypes included sporadic CJD (sCJD: n = 1999, 77%), familial CJD (fCJD: n = 398, 15%), Gerstmann-Sträussler-Scheinker syndrome (GSS: n = 99, 4%), fatal familial insomnia (n = 4), dura mater graft-associated CJD (dCJD: n = 86, 4%), variant CJD (n = 1), and unclassified CJD (n = 7).

The mean age of disease onset was 69 y in sCJD, 72 year in fCJD, 54 year in GSS, and 58 year in dCJD.

A total of 2061 deaths were reported, with a mean duration from onset to death of 19 months, ranging from 16 months in sCJD to 63 months in GSS.

A total of 149 patients with dCJD were registered, with a mean incubation period of 13 year and a maximum of 30 year.

In Japan, the numbers of patients with hPrDs tends to increase annually, indicating that older patients with rapidly developing dementia are increasingly being diagnosed with hPrDs by domestic physicians appropriately, supported by the CJD Surveillance Committee.

P-130: Diagnostic significance of Periodic synchronous discharges in Japanese surveillance of Creutzfeldt-Jakob disease

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The electroencephalographic finding of periodic synchronous discharges, PSDs or periodic sharp wave complexes, PSWCs is a basic clue for diagnosing CJD, along with diffusion-weighted MRI, and biochemical markers of 14-3-3 and tau proteins. Generalized periodic EEG patterns consist of 4 subtypes, periodic suppression bursts seen in cerebral anoxia and general anesthesia, periodic slow wave complexes seen in SSPE and ketamine anesthesia, repetitive sharp transients seen in CJD and cerebral anoxia, and periodic tri-phasic waves seen in metabolic encephalopathy. The PSDs in CJD correspond to the repetitive sharp transients. The occurrence rate of PSDs in Japanese CJD surveillance (2010 – 2011) was 63 percents in total 446 CJD patients, 76 percents in 339 sporadic CJD patients, 19 percents in 100 genetic CJD patients, and 85 percents in 7 dura CJD patients. Among 100 genetic CJD patients, the

occurrence rate of PSDs was 0 percent in 53 V180I mutation CJD patients, 9 percents in 22 P102L mutation CJD patients, 62 percents in 8 E200K mutation CJD patients, and 70 percents in 10 M232R mutation CJD patients. We proposed grading of PSDs in CJD, Grade A, B, C, D, and E. Grade A was defined as typical PSDs. Grade B was defined as PSDs of a relatively longer periodic interval. Grade C was defined as PSDs of a relatively rare appearance. Grade D was defined as PSDs of a rudimentary appearance. Grade E was defined as absent PSDs. Diagnosis in non-CJD patients registered in our CJD surveillance included status epilepticus, diffuse Lewy disease, Hashimoto encephalopathy, Wernicke encephalopathy, uremic encephalopathy, and lateral sinus thrombosis.

P-131: Creutzfeldt-Jakob disease associated with a V203I homozygous mutation in the prion protein gene

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A 73-year-old woman developed rapidly progressive gait disturbance and cognitive dysfunction. Three months after the onset, she became bedridden state. On admission to our hospital, neurological examinations revealed severe cognitive impairment and left-sided hemiparesis. Diffusion weighted brain magnetic resonance imaging showed hyperintensity in the right basal ganglia and the right frontal, parietal, and occipital lobes. Cerebrospinal fluid study revealed an elevation of tau protein and 14-3-3 protein. Gene analysis revealed a homozygous V203I mutation in prion protein gene (PRNP). The polymorphisms of the PRNP showed methionine homozygosity at codon 129 and glutamine homozygosity at codon 219. Four months after the onset, she entered a state of akinetic mutism accompanied by frequent myoclonus; moreover, periodic synchronous discharges appeared on the electroencephalography. We thus made a diagnosis of familial Creutzfeldt-Jakob disease (CJD) with a V203I homozygous mutation. The genotype frequency of the V203I mutation in the genetic prion diseases has been reported as 0.9% in the Japanese (2/216 cases) and 1.2% in Europeans (5/425 cases). All previously reported cases of familial CJD with the V203I mutation had a heterozygous mutation showing manifestations similar to those of typical sporadic CJD. There have been no reports of familial CJD with the V203I homozygous mutation. Although genetic prion diseases with homozygous PRNP mutations often present with an earlier onset and more rapid clinical course than those with heterozygous mutations, no difference was found in clinical phenotype between our homozygous case and reported heterozygous cases.

P-132: Clinical features in the patients with V180I, M232R and P102L of PRNP

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Approximately 20% of prion diseases in Japan are associated with mutations in the prion protein (PRNP) gene. We hereby report a case with familial Creutzfeldt-Jakob disease (fCJD) V180I, a case with fCJD M232R and 2 cases with Gerstmann-Straussler disease (GSD) P102L. These gene mutations are characteristically seen in Japan.

Case 1 was 85-year-old woman with V180I. She had no family history. She showed hallucinations, delusions and tremor on the left hand at 84 y old. The DWI image of brain MRI showed high intensity signal in the cerebral cortex, caudate nucleus and putamen. She became akinetic mutism 15 months after onset. Case 2 was 78-year-old woman with M232R. She had no family history. She noticed visual impairment, gait disturbance, and pain of left leg when she was 77 y old. The DWI image of brain MRI showed high intensity signal in the occipital cortex. She became akinetic mutism 4 months after onset and died 5 months later. Case 3 was a 61-year-old woman with GSD. When she was 47 y old she showed nystagmus, truncal ataxia, and pyramidal tract signs but no dementia. Her symptoms were similar to Machado-Joseph disease (MJD). DWI brain MRI showed no abnormality during the early stages. She developed akinetic mutism 9 y after onset and died 5 y later. Case 4 was the older brother of case 3. He noticed painful dysesthesias of his lower limbs at 59 y old. 2 y later he showed truncal ataxia and mild dementia. He developed akinetic mutism 6 y after onset and died 1 y later. The pathological findings of case 4 showed spongiform changes in the cerebral cortex and amyloid-like plaques in the cerebellum. We detected P102L mutation in the PRNP.

The most common phenotypes with V180I are slowly progressive cognitive impairments similar to Alzheimer's disease. The patients with M232R show typical sporadic CJD. The patients with P102L onset at 50s, and mimic SCD. Their DIW images of brain MRI show no abnormality.

Because phenotypes of hereditary prion diseases are varied, the PRNP gene screening is useful for progressive neurodegenerative diseases.

P-135: The Japan Consortium of Prion Disease (JACOP) for patients' registration and clinical studies of Prion diseases in Japan

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JACOP (Japanese Consortium of Prion Disease) was established for registration of Prion disease patients and elucidation of natural courses of Prion diseases, which seems essential to any clinical researches of Prion diseases including clinical trials in future.

The secretariat office has been settled in National Center Hospital, National Center of Neurology and Psychiatry (N.C.N.P) since 2015.

The number of institutions participating in JACOP has reached 100 in Feb. 2016. The number of doctors who have joined JACOP is over 250 in Feb. 2016.

The registration and natural history research of Prion diseases started in 2014. Doctors perform medical interview and examination of patients once in 3 months, and Clinical

Research Coordinators do telephone-interviews of patient's families who give the care of patients or to the physician in charge once in a month.

In this natural history research, we use a Japanese version of MRC-rating-scale which was adapted in PRION 1 study and others.

Until Feb. 2016, 35 patients has been observed their progress in the natural history research. The 9 out of 35 patients died in the observing period. Most of patients have become the akinetic state in the early period of observation.

We have tried to enroll patients of their early stages to make the research more meaningful. We sent mails to all the Board-Certified Neurologists of the Japanese Society of Neurology (JSN) and to the Directors of the Education Hospitals of JSN.

NCNP began to support other institutions to speed up the reviewing process of the ethics committee of each institute.

We also prepared a registration system by the patients or their families themselves in addition to the classical institutional registration mentioned above.

In this way of participation, a patient or the family can reach the document which they need, such as ID, questionnaire forms and schedule of the clinical research through the web-site of JACOP. Such patients need of course many helps from thier doctors to fill in the questionnaire forms.

We curently plan to integrate the surveillance process by the CJD Surveillance Committee and registration processe by JACOP so that we would be able to reduce doctors' burden and increase numbers of participated patients.

P-137: An early glimpse of saturation mutagenesis in humans: Insights from protein-coding genetic variation in 60,706 people

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Every human harbors an average of ~60–70 de novo DNA mutations. Given the world population of 7 billion and the ~3 billion base pair human genome, this implies that every possible single nucleotide variant has, on average, arisen independently in tens or hundreds of people worldwide. With data from 60,706 humans, the Exome Aggregation Consortium (ExAC) dataset has already reached a size where we can directly observe evidence of widespread mutational recurrence - the same genetic variant arising independently in different people - and where the most frequent mutation types are nearly saturated. This talk will discuss 3 different implications for the study of neurodegenerative diseases. First, we can infer the strength of natural selection acting upon each gene by observing constraint, the conspicuous depletion of protein-altering variants. Second, through "genotype-first" ascertainment we can perform reverse genetics in humans, learning whether disruption or modification of a given gene has any phenotypic effect, providing validation for proposed drug targets. Third, by combining allele frequency and mutation type information, we can make educated inferences about whether new genetic variants seen in neurodegenerative disease patients are causal or coincidental.

P-138: Clinically and neuropathologically atypical autopsied case of sporadic Creutzfeldt-Jakob disease MM type1

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We report a Japanese patient of sporadic Creutzfeldt-Jacob disease (CJD) with atypical pathological findings. A 47-year-old woman developed amnesia and disorientation. Two months later, her family noticed the behavioral abnormality, and she was admitted to Kanto Central Hospital. Results of the detailed examination were highly suggestive of CJD. In the cerebrospinal fluid, total tau and 14-3-3 proteins were markedly elevated. Real-time quaking-induced conversion (RT-QUIC) test was positive. Electroencephalography revealed periodic synchronous discharge with flattened background activities. On MRI examination, cortical high intensity spread diffusely in the cerebrum on diffusion-weighted images. The symptoms were rapidly progressive; she could not converse in 2 months and myoclonus was present. She became akinetic mutism and died after 9 months clinical course.

On autopsy, the brain weighed 1,202g. Frontal sulcus was mildly enlarged. The cerebral cortex narrowed diffusely. In contrast, hippocampus and primary motor cortex were relatively preserved. Microscopic examination revealed marked neuronal loss with reactive gliosis and spongiform changes in cerebral neocortex. Deposition of prion protein (PrP) was extensive and mainly with synaptic patterns. Similar findings were observed in the basal ganglia and thalamus. The cerebellum was also affected with dense PrP deposition in the granular layer.

By molecular analysis, no pathogenic mutation was found on the PRNP gene, and the polymorphisms showed methionine and glutamine homozygosity at codon 129 and 219 respectively. Protease-resistant PrP detected by blot analysis was compatible with type1, however, the molecular weight of the non-glycosylated form of PrP^{Sc} was slightly lower than 21kDa.

The pathological findings were unusual as MM1 subtype in some points. In addition to the finding described above, white matter degeneration was conspicuous considering the disease duration. Perineuronal PrP deposition was noticeable in the hippocampus and amygdala. PrP deposition spread to substantia nigra accompanied by depigmentation.

Slight conformational abnormality of the PrP^{Sc} was speculated in this patient by the blot analysis, and it can correlate with the atypical pathological findings and also early onset of the disease.

P-139: How can we increase the number of prion autopsy in Japan?

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Autopsy is important in understanding the pathomechanism as well as in establishing treatments for prion disease. However, the number of autopsy carried out in Japan is small. According to the surveillance center of prion disease in Japan, the number of prion autopsy cases is less than 20 cases per year.

To increase the number of prion autopsy cases, the brain bank of Mihara Memorial Hospital has performed the following. 1) We have

a special ward for intractable neurological disorders and has actively admitted prion disease patients. 2) In cases of autopsy requests from other hospitals, our staffs on call around the clock engage in the transportation of bodies. The transportation of the bodies are financed by the hospital, therefore, we do not charge transportation fees to the patients' families. 3) The autopsy is carried out on call. 4) After finishing the autopsy, we transport the body to places such as homes and funeral homes as requested by the families.

We have carried out 40 autopsy cases in our hospital and 5 cases in other hospitals last 8 y. Autopsy was carried out according to the autopsy guideline of prion disease, and the right hemi-brain was stored at -80 celsius. The left hemi-brain was fixed using 20% buffered neutral formalin. The 19 histologic sections were stained using Hematoxylin and eosin, and Krüver-Barrera method. For immunohistochemistry, the sections were immunolabeled using antibodies raised against prion (3F4, Covance), amyloid-β (11-28), p-tau (AT8), p-α-synuclein, and GFAP on the Ventana automatic stainer. The frozen sections of the frontal cortex and cerebellum are sent to the surveillance system for molecular analysis.

Although most cases are sporadic CJD, we identified DCJD in one case, P102L in 2 cases, V180I in 2 cases, E200K in one case and M232R in 3 cases. Two cases that were clinically considered as cerebral infarcts and spinocerebellar ataxia were diagnosed as prion disease after the autopsy. In contrast, 3 cases were denied as having prion disease by autopsy. As for 2 out of the 3 cases, other hospitals refused to perform autopsy due to the possibility of prion disease.

Many hospitals and pathologists are unwilling to perform autopsy because of infectivity. However, we believe that prion autopsy is not a difficult technique along to the guideline. We need to enlighten neurologists and pathologists to carry out neuropathologic and molecular diagnosis of clinically suspected prion disorders.

P-140: "Wire-QuIC": A new detection system of human prion

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Accidental transmission of prions in neurosurgery has been reported as a consequence of re-using contaminated surgical instruments. Although several decontamination methods has been studied by using animal prions such as a hamster-prion 263K, there was no data directly evaluated human prions. A newly developed *in vitro* amplification system, designated RT-QuIC, has first enabled to assess the activity of abnormal prion proteins within a few days. RT-QuIC using human recombinant prion protein (PrP) showed high sensitivity for human prions as the detection limit of our assay was estimated as 0.1 fg of active prions. We applied this method for detection of human prion activity on stainless steel wire. When we put wires contaminated with CJD patient's brain directly into the test tube, the typical PrP-amyloid formation was seen within 48 h and we could detect activity of prion, 50% seeding dose (SD_{50}), on this wire from $10^{2.8}$ to $10^{5.8}$. Using this method, we also confirm that the seeding activities on wire were removed by treatment with 1N NaOH. As the seeding activity could be closely correlated to infectivity of prions in bioassay, this wire-QuIC assay will be useful in direct evaluation of the decontamination methods for human prions.

P-141: The first Italian case of Creutzfeldt-Jakob disease with V180I mutation in the PrP gene (PRNP)

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Creutzfeldt-Jakob disease (CJD) is an invariably fatal brain disorder. The majority of cases manifest as sporadic and only the 10-15% represent the genetic form. The Italian surveillance reported since 1993 more than 444 cases with different point and insert mutations. Here we report the first Italian CJD case linked to a rare mutation in the prion protein gene (PRNP) leading to an amino acid substitution from valine (V) to isoleucine (I) at codon 180 (V180I). The patient was a 74-year-old man who, developed psychiatric symptoms (mood depression, insomnia, apathy, abulia and withdrawal) after a period of emotional stress and wastreated with antidepressants without benefit. Within the first month the psychiatric symptoms worsened with association of forgetfulness, ideomotor apraxia, disphasia, agraphia, ataxic gait with lateropulsion and falls. Seven months later the patient was hospitalized because of a rapidly progression of the cognitive and motor symptoms. On admission, the patient's neurological examination showed a reduced psychomotor ability, mild hypofonia, dysmetria of the upper and lower limbs on execution of the cerebellar tests, and a tendency to retro-lateropulsion. The gait was possible only with bilateral support. He presented also urinary incontinence. The MMSE performed in that period was 9/30. Blood tests including paraneoplastic markers assessment, thyroid hormones, vitamin B12, tumor markers, cerebellar auto-antibodies, were normal. Repeated EEG recordings were performed, showing diffuse slowing with generalized theta-delta activity, without a typical periodism. Brain MRI, on FLAIR sequences, revealed a symmetric hyperintensity of the nucleus caudatus head and putamen; the cortical fronto-parieto-temporo-occipital regions were also involved. During hospitalization the patient developed myoclonus of the upper limbs, extrapyramidal and pyramidal signs and global aphasia. CSF analysis showed a negative 14-3-3 proteins test. Real Time Quaking Induced Conversion (RT-QuIC) assay was performed to detect the presence of the pathological misfolded prion protein PrP on CSF and olphactory epithelium (OE) collected by Nasal Brushing. Both tests were negative. The PRNP genotyping evidenced the V180I mutation, while the polymorphism at codon 129 resulted in methionine/valine heterozygosity. A diagnosis of probable genetic CJD was made according to International criteria. The patient died 35 months after the first symptoms. An autopsy limited to the brain was performed. The patient's past medical history was overall unremarkable apart from one surgical intervention of nefrectomy and one cholecystectomy. There was positive family history for neurodegenerative diseases. Family members requested genetic counseling and agreed to genetic testing.

P-142: Evaluation of CSF RT-QuIC diagnostic assay for Creutzfeldt-Jakob and other human prion diseases: The Italian Surveillance Unit experience

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Background. The introduction of CSF 14-3-3 analysis in the diagnostic criteria for sporadic CJD significantly increased the sensitivity of

diagnosis. However, CSF 14-3-3 test is based on surrogate markers of neuronal damage or death that is not linked to the primary molecular pathology of prion disease. Recently the Real Time-Quaking Induced Conversion (RT-QuIC) test, developed for detection of prion seeding activity, has been shown to detect the abnormal prion-disease specific PrP^{Sc}, including all subtypes of sporadic CJD, down to femtogram levels. In this study we aimed to compare the sensitivity, specificity, repeatability and reproducibility of the CSF RT-QuIC test with existing diagnostic methods for human prion diseases.

Method. RT-QuIC assay is based on PrP^{Sc} seeded polymerization of bacterially expressed recombinant prion protein (the substrate) in multiwell plates, with the resulting aggregated protein being detected on a Omega Plate reader using an amyloid sensitive dye, the thioflavin T, according to published protocol (Imbriani et al 2015).

Study population. Study population was composed of 211 patients referred to the Italian Registry (70 definite, 26 probable, 5 possible sporadic CJD and 11 genetic CJD, plus 99 non CJD controls). Information from each patient was collected including age, sex, duration of the disease, MRI, EEG, PRNP gene sequencing data for disease-causing mutations and codon 129 polymorphisms, neuropathological classification, and levels of CSF surrogate markers 14-3-3 and total tau proteins whenever possible.

Results. The results of CSF RT-QuIC were compared to other diagnostic information available for the same test cases in order to assess the relative merits of various tests, including RT-QuIC, that are used in the clinical diagnosis of different phenotypes and genotypes of human prion diseases. The primary advantage of RT-QuIC test is that it provides an etiological diagnosis based on the detection of PrP^{Sc}. We obtained a sensitivity of around 84% and a specificity of 94%. If we consider both tests (RT-QuIC and 14-3-3) we increase such sensitivity even if we still loose around 3% sporadic CJD patients.

Conclusion. PrP^{Sc} detection in the CSF by RT-QuIC represents a potential disease specific laboratory test allowing a more accurate clinical diagnosis of sporadic CJD.

P-143: A case of slowly progressive familial prion disease with a 5-octapeptide repeat insertion

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We report a 41-year-old woman of familial prion disease with a 5-octapeptide repeat insertion (OPRI) in the prion protein gene, whose clinical course were different from her father who had same mutation.

She noticed that she could not write in large characters and not recall letters well. One year later, her balance became worse and she often felt from a bicycle. She had difficulty in calculating, memorizing and using cash register during her job. Her mother noticed her behavioral abnormalities in daily living and brought her into our hospital.

She had no past history, abuse and regular medications. She had never been to United Kingdom. Her father showed dementia and gait disturbance at the age of 52-year old. His symptoms had progressed so rapidly that he had been diagnosed as having Creuztfeldt-Jakob disease. Myoclonus appeared 2 month later followed by akinetic-mutism, and died after 3 months clinical course. Autopsy and genetic testing revealed familial prion disease with 5-OPRI. His brother had same diagnosis and showed similar clinical course.

She was courteous and cooperative. Her mini-mental state examination (MMSE) score was 20 because of disorientation in time and difficulty in calculating, writing a composition

and drawing pentagons. She showed mild cerebellar ataxia and wide based waddling gait. Her deep tendon reflexes were all increased symmetrically and Chaddock reflexes were seen. No parkinsonian signs were apparent except for induced rigidity. Myoclonus and startle reflex were not seen.

Brain MRI showed generalized atrophy without abnormal intensity in any region. Total tau and 14-3-3 proteins in the cerebrospinal fluid were both within normal ranges. Electroencephalography (EEG) did not show any paroxysmal discharge including periodic synchronous discharge (PSD). The DNA analysis revealed 5-OPRI in the prion protein gene.

Her symptoms were slowly progressive. Three years later, parkinsonian symptoms such as tremor and cog-wheel regidity became apparent. Cerebellar ataxia had worsened but she could walk by herself. Four years later, her cognitive function had worsened and MMSE score was 14. She sometimes got lost. She needed to use wheel chair because of worsening of parkinsonian symptoms, ataxia and limb-kinetic apraxia. Myoclonus was not apparent and EEG did not show PSD.

We report this unique case because her phenotype is quite different from her father's regardless of the same genotype. It is suggested that not only the genetic mutation, but also some other factors may influence the clinical course of familial prion disease with OPRI.

P-144: Human Prion Diseases Surveillance and registration system in Japan

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A nationwide surveillance and registration system for human prion diseases (hPrDs) in Japan has been operated since April 1999, by the Creutzfeldt-Jakob Disease (CJD) Surveillance Committee. We hold the committee twice a year and discuss about clinical, familial, and social (occupation, abroad history and so on) information of all the patients.

The information of patients suspected of hPrDs is obtained from 3 sources. (1) the MHLW (ministry of health, labor and welfare)' s Project on Overcoming Intractable Diseases, (2) notifications under the Infectious Diseases Control Law(IDCL), (3) requests from physicians to the CJD Surveillance Committee to take genetic or cerebrospinal fluid (CSF) analysis at Tohoku University and Nagasaki University, respectively. Among three sources, the leading is third one (from genetic and CSF analysis laboratories). In 2015, the number of cases discussed in the Committee was 387, containing duplicating resources, i.e. 58 cases from MHLW, 29 cases from IDCL, 326 cases from Nagasaki University and 120 cases from

Tohoku University, and 19 cases from other root. The contribution of these universities to CJD Surveillance.

We hope to make the surveillance and registry as a complete enumeration inventory survey, but a falling rate of hPrDs is estimated to about 10-20%. An autopsy rate of hPrDs in Japan is 16%. This low rate is from Japanese feeling the autopsy as desecration of the dead and from a small number of anatomy facilities for hPrDs.

To progress the research and treatment of hPrDs, we need to promote our registration system in more detail and raise the autopsy rate.

P-146: RT-QUIC detection in CWD infected cervid and TgElk mice tissues

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Transmissible spongiform encephalopathy (TSE) is a fatal neurodegenerative disorder, which is so-called as prion diseases due to the causative agents (PrP^{Sc}). Chronic wasting disease (CWD) agents are shed in blood, urine and feces which most likely contribute to the horizontal transmission between cervid species. The body fluids of TSE-infected animal using conventional detection methods are difficult to be proven the presence of PrP^{Sc}. The development of amplification-based seeding assay has been instrumental in detection of low levels of infectious prions in clinical samples. Using real-time quaking-induced conversion (RT-QUIC), we established a detection method for (CWD) PrP^{Sc} in brain tissue of CWD affected cervid and CWD infectious sequential study in transgenic mice overexpressing elk prion protein (TgElk mice) model. We found that PrP^{Sc} was detected at extremely low levels (2X10-13 dilution). PrP^{Sc} was detected in the brain and blood from 30dpi. We established the modified RT-QUIC including high reaction temperature and rapid shaking. Our method appears to be a very useful technique for measuring prion contamination of organs, blood and food products.

P-147: Infection and detection of PrP^{CWD} in soil from CWD infected farm in Korea

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Transmissible spongiform encephalopathy (TSE) is a fatal neurodegenerative disorder, which is so-called as prion diseases due to the causative agents (PrP^{Sc}). TSEs are believed to be due to the template-directed accumulation of disease-associated prion protein, generally designated PrP^{Sc}. Chronic wasting disease (CWD) is the prion disease that is known spread horizontally. CWD has confirmed last in Republic of Korea in 2010 since first outbreak of CWD in 2001. The environmental reservoirs mediate the transmission of this disease. The significant levels of infectivity have been detected in the saliva, urine, and feces of TSE-infected animals. Using serial protein misfolding cyclic amplification (sPMCA), we developed a detection method for CWD PrP^{Sc} in soil from CWD affected farm in 2010. We found to detect PrP^{Sc} in soil from CWD infected farm, but not detect PrP^{Sc} in soil of wild cervid habitats and normal cervid farm in Korea. We also tried the bioassay on transgenic mice overexpressing elk prion protein (TgElk mice) to confirm infectivity of CWD-infected farm soil and washing solution of it. As the results, there was the presence of infectious prions in them. The attack rates were each 12.5% (1/8, soil) and 100% (6/6, soil washing solution). Our method appears to be a very useful technique for monitoring PrP^{Sc} levels in environmental conditions.

P-148: Prion treatment by electrolyzed alkaline water and other chemicals

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Prion agents are known to be highly resistant pathogens. Therefore, inactivation of prions requires appropriate treatment of surgical instruments used for example in craniotomy, spinal surgery and ophthalmologic procedures. The most important difference between prion and other pathogens is that prion has no associated nucleic acid. Therefore, it cannot be inactivated by conventional sterilization procedures such as autoclaving (121C, 20 min), exposure to UV or gamma-ray irradiation. Inactivation of prions involves use of an autoclave under severe conditions (134C, 18 min), NaOH (1 N, 20C, 1 h), SDS (30%, 100C, 10 min), and NaOCl (20,000 ppm, 20C, 1 min).

In contrast to other pathogens like viruses and bacteria, fixation using aldehyde slightly decreases the prion titer but this is insufficient. Therefore, for treatment of tissue sections of prion infected animals, formic acid is recommended. Recently, the effectiveness of other treatments has been reported. Nonetheless, animal bioassays must be performed in order to fully demonstrate the effectiveness of these treatments on prion inactivation, which takes a considerable length of time (about 1 y or more). This makes it difficult to examine other forms of treatment given the cost and time-consuming nature of the tests. Currently, the following procedures for prion inactivation are recommended by the Japanese government and Society for Healthcare Epidemiology of America. (i) washing with appropriate detergents + SDS treatment (3%, 3-5 min), (ii) treatment with alkaline detergents (80-93C, 3-10 min) + autoclaving (134C, 8-10 min), (iii) washing with appropriate detergents + autoclaving (134C, 18 min), and (iv) washing with alkaline detergents (at a concentration and temperature according to instructions) + vaporized hydrogen peroxide gas plasma sterilization. Most importantly, a dried prion-infected apparatus is difficult sterilize, therefore prompt washing is essential. This procedure should be followed by autoclaving at 134C and vaporized hydrogen peroxide gas plasma sterilization in order to attain an assurance level of prion inactivation of less than 10^{-6}. Recently we have developed new way of treatment using functional water (Micro-cluster water). We can electrolyze water using only minerals ($CaHCO_3$) and create pH 12.5 alkaline water, which kills many kinds of pathogens including multi-drug resistant bacteria and prions. In the future we may apply it to humans, animals or food directly.

Acknowledgments. Those works were supported by Dr. Masayoshi Yukawa in Nihon University, Dr. Akikazu Sakudo in Ryukyu University and Dr. Takeshi Haga in University of Tokyo.

P-149: Key features of the Australian BSE food safety assessment process

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In response to the BSE outbreak, the EU, Argentina, Australia, Japan, Singapore, the United States, and the World Organization for Animal Health (OIE) developed various BSE country categorisation systems. The geographical BSE risk assessment system developed by the EU was considered the model and since 2007 the OIE's categorisation system, focusing on entry and exposure assessment has been the internationally accepted process of defining a country's BSE risk status for animal health.

The BSE food safety assessment process developed and implemented by Food Standards Australia New Zealand as part of the implementation of Australian Government's revised BSE food safety policy since 2010 is based on the OIE assessment system. Australia's BSE food safety assessment however has included additional elements that extend the categorisation of BSE risk status to food safety. The key

features of the Australian BSE food safety assessment process include:

A clear description of the BSE prevention and control system in the country as represented by: (1) an illustration of the country's legislative instruments to prevent the introduction and spread of BSE; (2) the structure of governmental tiers and administrative arrangement for BSE prevention and control; and (3) a systematic assessment of the BSE prevention and control measures implemented in the country from an assessment of the potential for introduction of the BSE agent through imports of live cattle and bovine products, to an assessment of the effectiveness of the ruminant feed ban, beef production system, traceability system for cattle and beef, together with an assessment of BSE surveillance in the country;

The assessment is driven by data, information and evidence where considerable effort is devoted to demonstrate the effectiveness of the country's BSE prevention and control measures through the provision of data on imports, parameters of inspection and audits across the beef supply chain, and surveillance data;

A focus on traceability assessment which assesses the cattle identification and traceability system and food recall system used in the country;

An emphasis on observing and verifying the effectiveness of controls implemented through a verification inspection along the beef production chain in the country;

Flexibility in dealing with how systems are implemented differently by countries; and

Transparency in communicating the risk assessment process and findings.

These features are applicable to not only the assessment of country BSE status, but also the assessment of a country or region's risk status impacted by other types of zoonotic diseases.

P-150: Study of the allelic variants at codon 222 in the genome goat for the detection of susceptibility to SCRAPIE, through the mass spectrometry (MALDI-TOF)

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In sheep, the susceptibility to transmissible spongiform encephalopathies (TSE) is strongly modulated by polymorphisms of the prion protein (PrP) gene and the nature of the prion disease agent. The A136 R154 R171 allele is associated with a highly protective effect against natural or experimental infection with classical scrapie and bovine spongiform encephalopathy (BSE) agents, while the V136 R154 Q171 or A136 R154 Q171 alleles are associated with susceptibility. At the European level, the selection of the ARR allele carriers was successfully applied for controlling and eradicating classical scrapie in infected sheep flocks. At the population level, large-scale selection programmes were also implemented. They aimed at increasing the frequency of the ARR allele in the general population making it less favorable for TSE agent circulation and spreading. This 'breeding for resistance policy' in combination with the other eradication measures, resulted in a significant reduction of the classical scrapie prevalence in populations where it was comprehensively applied. In goats, several field studies have identified that the coding mutations 222 of the PrP gene is associated an high resistence to developing classical scrapie. The development of a PrP genotype selection program is now considered by the EU authorities as a potential tool for the control and eradication of scrapie in the commercial goat population. The objective of this study was to evaluate the MALDI-TOF mass spectrometry for analysis of polymorphisms in

the genome goat for the detection of susceptibility to SCRAPIE. MALDI-TOF mass spectrometry is now being used for analysis of nucleic acids, including genetic variations such as microsatellites, insertion/deletions, and, especially, single nucleotide polymorphisms (SNPs). The output data are a measure of an intrinsic characteristic of the DNA products being studied (molecular weight in Daltons); no indirect measurement of the products is involved, as with fluorescent or radiolabel tagging. The ability to resolve oligonucleotides varying in mass by less than a single nucleotide makes MALDI-TOF mass spectrometry an excellent platform for SNP and mutation analysis. A highly automated processing platform incorporating MALDITOF mass spectrometry, designated DNA MassARRAY, has been developed. DNA MassARRAY uses samples in chip-based, high density arrays. This system accurately calls SNPs in individual DNA samples, or alternatively determines SNP allele frequencies in DNA pools. Assay design for MassARRAY is simple, flexible and has been automated to allow designing many assays, all of which can be run using a universal set of reaction conditions.

P-151: Phenotypic plasticity of chronic wasting disease prions

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Among transmissible spongiform encephalopathies (TSEs, prion diseases), chronic wasting disease (CWD) is unique and especially problematic because it affects free-ranging wildlife, can be facilely transmitted, and is caused by an agent that persists in the environment. These factors have contributed to the aggressive spread of CWD in North American deer and elk populations with as-yet unknown disease risks to other species exposed to CWD prions. Prions lack nucleic acids, limiting the tools available to discriminate among strains circulating in CWD-affected cervid (deer, elk, and moose) populations. Here, we show that the meadow vole (*Microtus pennsylvanicus*), a native North American rodent sympatric with existing CWD epizootics, is susceptible to CWD and can be used to characterize CWD prion strain diversity in brain tissues from wild white-tailed deer (WTD; *Odocoileus virginianus*). Meadow voles manifest at least 4 distinct disease phenotypes following intracerebral challenge with CWD isolates from WTD heterozygous for glycine/serine at residue 96 of the prion protein (GS^{96}-CWD), a polymorphism that influences CWD susceptibility in WTD. In contrast, challenge with WTD CWD isolates homozygous for glycine (GG^{96}-CWD) yields a single phenotype. Notably, intraperitoneal challenges with GG^{96}-CWD result in a disease phenotype distinct from that produced by intracerebral challenge, demonstrating that exposure route influences CWD strain selection in meadow voles. Disease phenotypes produced by GS^{96}-CWD isolates are subject to phenotypic switching during subsequent passages, while GG^{96}-CWD phenotypes appear fixed. We find that a GS^{96}-CWD isolate can cause subclinical infection in voles following oral challenge that manifests as clinical disease upon further passage. Our cross-species transmission experiments reveal the existence of a mixture of CWD strains in WTD isolates from wild populations and implicate a role for cervid host PrP genetics in CWD strain selection in WTD. Furthermore, we identify meadow voles as useful tools for CWD strain typing.

P-152: Sensitive and rapid diagnosis of goat prion diseases by 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, 2 natural BSE cases have been reported in goats. Furthermore, experimental transmissions of classical (C-BSE) and atypical form of BSE (BASE) in goats were also reported. On this basis, the development of a new approach for ante-mortem diagnosis and characterization of small ruminant TSE strains may be a feasible target that would help to reduce the risk of epidemic spread of goat TSE.

The aim of this study was to investigate the use of the Real Time QuIC (RT-QuIC) assay for the diagnosis of TSEs in goats. We evaluated the sensitivity and specificity of this technique for the detection of prion seeding activity in brain tissues and cerebrospinal fluid (CSF) from animals naturally and experimentally infected with Scrapie or bovine isolates of C-BSE and BASE.

Our findings indicated that by using the Ha-S rPrPSen substrate, the RT-QuIC can sensitively detect both PrP^{Sc}rapie, PrP^{C}-BSE and PrPBASE associated seeding activity in goat brain tissues in less than 48 hours. Furthermore, our ability to detect 108-fold dilutions of all goat prion strains-infected brain tissues indicates that the RT-QuIC is at least as sensitive as infectivity bioassays. To further optimize the conditions, we examined the effects of temperature, as well as of the concentrations of sodium dodecyl sulfate (SDS) on RT-QuIC reactions using CSF as seed. The average fluorescence increase was stronger and faster for the BASE-infected CSF, showing positive signals over the control samples as early as 10 h, compared to the 40 h it took for the reactions in SDS-free conditions. Therefore we chose to use SDS in subsequent analyses. All goat PrP^{Sc} strains were rapidly detected in CSF using new improved RT-QuIC protocol conditions and rHaSPrPSen 23-231 or rHaPrPSen 90-231 as substrates.

Taken together these data are indicative of the great potential of these *in vitro* prion amplification assays for the ante-mortem diagnosis of prion diseases in small ruminants. We have preliminary experimental evidence showing that RT-QuIC sensitively and specifically detects prion seeding activity in cerebrospinal fluid from symptomatic goats.

P-154: Prion infectivity detected in swine challenged with chronic wasting disease via the intracerebral or oral route

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Chronic wasting disease (CWD) is a naturally-occurring, fatal neurodegenerative disease of North American cervids. The potential for swine to serve as a host for the agent of chronic wasting disease is unknown. In the US, feeding of ruminant by-products to ruminants is

prohibited, but feeding of ruminant materials to swine, mink, and poultry still occurs. In addition, scavenging of CWD-affected cervid carcasses by feral pigs presents a potential risk for CWD exposure. The purpose of this study was to investigate the susceptibility of swine to the CWD agent following oral or intracranial experimental challenge.

At 8 weeks of age, crossbred pigs were challenged by the intracranial route (n = 20), oral route (n = 19), or were left unchallenged (n = 9). At approximately 6 months of age, the time at which commercial pigs reach market weight, half of the pigs in each group were culled (<6 month challenge groups). The remaining pigs (>6 month challenge groups) were allowed to incubate for up to 73 months post challenge (mpc). At death a complete necropsy examination was performed, including testing of tissues for misfolded prion protein (PrPcwd) by western blotting (WB), enzyme-linked immunosorbent assay (ELISA), and immunohistochemistry (IHC).

None of the pigs developed clinical signs consistent with prion disease. Four >6 month intracranially challenged pigs (survival times 45-73 mpc) were positive by ELISA, 2 were also positive by WB, and one was positive by IHC. One >6 month orally challenged pig (64 mpc) was positive by ELISA.

To further investigate the potential for infectivity, brain tissue from selected pigs was bioassayed in mice expressing porcine PRNP. Tissue from the 2 WB-positive >6 month intracranially challenged pigs produced positive bioassay results, albeit with low attack rates and variable incubation periods. Interestingly, bioassay of material from the longest surviving >6 month orally challenged pig (72 mpc), which was negative for PrPcwd by all other tests, produced a positive bioassay result. Bioassay of material from additional animals is currently underway.

This study demonstrates that pigs can serve as potential hosts for CWD, although with low attack rates and scant PrPcwd accumulation. Detection of infectivity in orally challenged pigs using mouse bioassay raises the possibility that naturally exposed pigs act as a reservoir of CWD infectivity, even though affected pigs do not develop overt clinical signs or readily detectable PrPcwd.

P-155: Oral transmission of classical BSE to adult cattle

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Despite strict measures to eradicate classical bovine spongiform encephalopathy (cBSE), numerous countries around the world continue to detect residual cases. This invariably fatal, neurodegenerative disease of cattle is believed to be transmitted by the consumption of contaminated feed early in life. To test whether older animal are susceptible to BSE infection, we fed adult cattle a large dose of cBSE positive brain and allowed them to incubate until the onset of clinical disease. At the presentation of clinical signs, animals were euthanized, sampled and tested for BSE.

At 48 months post challenge, all 3 cattle orally challenged with cBSE started to show initial clinical signs and were euthanized at 53 months post challenge. When tested, 2 of the animals were strongly positive but the third animal tested negative on BSE rapid screening tests; indicating that adult cattle are susceptible to oral transmission of cBSE, albeit with a slightly extended incubation period.

Additional testing of this third animal subsequently detected perivascular immunohistochemical reaction in the frontal cortex but protein misfolding cyclic amplification was the only method to indicate the presence of BSE in its brain stem. To find an explanation for this abnormal result, the genetic background of all 3 cattle were further investigated. Genetic sequence was generated for approximately 4000 base pair preceding the Prnp open reading frame. This allowed us to determine that the abnormal animal had a homozygous genetic

deletion near the Prnp gene on chromosome 13. This deletion might have an impact on cellular prion protein expression or may be linked to other genetic anomalies that affect BSE. In addition, the same genotype was present in 2 animals orally challenged with atypical H and L type BSE which were displaying clinical signs but also tested negative on rapid screening tests. These results point toward the existence of a genetic modification with a significant impact on BSE susceptibility and/or presentation.

P-156: Detection of prion protein specific camelid nanobodies: Implications for prion disease therapeutic options

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B-lymphocytes produce highly specific heterotetrameric antibodies when challenged with antigen. Most species produce antibodies composed of 4 polypeptides, which consist of 2 heavy chain domains and 2 light chain domains. Each light chain domain contains a variable region and a hyper-variable region, which allow for specific contact interaction with the antigen by means of differing amino acid sequencing. However, Camelid sera contains both heterotetrameric antibodies as well as functional homodimeric antibodies that are devoid of variable regions and derived from the heavy-chain variable (VHH) domains on Immunoglobulin (Ig) molecules. These smaller antibodies, termed nanobodies, are smaller than the heterotetrameric antibodies found in other species. They are the cloned portion of the Complimentarity Determining Region of the single chain variable fragment, and have implications for treatments due to the reduced size and antigen specificity. After seven immunizations utilizing white tailed deer recombinant PrP as the antigen, specific antibodies have been detected using Enzyme Linked Immunosorbent Assays. Post immunization bleeds 4, 5, 6, and 7 show a statistically significant increase in optical density (O.D.) when contrasted to numerous negative controls. A threshold was determined by taking the average O.D. of the negative controls plus 3 standard deviations above the mean. Positive sample sera contained an antibody titer between 2,000 and 4,000, indicating that the antibody that is being identified within this assay is specific for the antigen of white tailed deer PrP. This project focuses on the production, the specificity, and the future implications of Camelid nanobodies within prion disease.

P-157: Earthworms can act as carriers for prion disease transmission

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Chronic Wasting Disease (CWD) is a prion disorder affecting captive and free-ranging deer and elk. CWD efficiently propagates by horizontal transmission and compelling evidence suggests a potential role of environmental contamination. Infectious prions may enter into the environment through carcasses from diseased animals, saliva, feces, urine, blood or placenta tissue from clinical or preclinical infected animals. Recent studies have shown that infectious prions bind tightly to soil, plants and other environmental components, remaining infectious for long periods of time.

We hypothesize that earthworms, which get in contact with infectious prions through contaminated soil, may play a role on the horizontal transmission of prion diseases. To study whether worms can bind and retain prions, we analyzed red wiggler earthworms exposed to soil contaminated with 263K hamster prions. Studies were done *in vitro* using the PMCA technique and *in vivo* in Syrian hamsters. For *in vitro* analyses, the worms were placed for 1, 3, 7, 14 and 28 d in contaminated soil, washed thoroughly, homogenized and analyzed for the

presence of PrP^{Sc} by PMCA. The results show that even worms exposed for only one day to contaminated soil can bind PrP^{Sc} and sustain the conversion of normal prion protein. Furthermore, in order to determine if worms might be able to transport prions in the soil, we placed contaminated worms in uninfected soil and analyzed the presence of PrP^{Sc} in the worm and the soil over time. Our data show that worms release PrP^{Sc} into the soil and in this way acting as carrier of infectious materials from one place to another.

For *in vivo* studies, hamsters were intraperitoneal infected with homogenates from worms that were growth in prion infected soil for 28 d. Hamsters, inoculated with 263K-contaminated worms, developed typical signs of prion disease, whereas control animals inoculated with non-contaminated worms did not. Prion disease was confirmed by biochemical and histological analyses.

Overall, our results suggest that earthworms exposed to infectious prions through contaminated soil, can effectively bind, retain and transport PrP^{Sc}, and thus may act as carrier of infectivity and possibly play an important role for horizontal prion transmission.

P-159: The epidemiological evolution of prion infection on bovine in Romania, in the period of 2010-2015

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The first BSE epidemic began in England in 1986 and progressed steadily until 1992. Implementation of preventive measures materialized by progressive decline of the disease but new cases continued to occur in different European countries.

This prion disease in Romania was first diagnosed in 2014, at the Institute for Diagnosis and Animal Health, in the National Reference Laboratory for transmissible spongiform encephalopaties.

The epidemiological evolution of BSE in cattle are presented in this paper, with methods used, the results of investigations, the outbreaks and the number of cattle diagnosed with this prion disease.

Were subjected to epidemiological investigations and laboratory tests 510166 large ruminants in the period of 2010 -2015.

The whole diagnostic activity was coordinated by the National Reference Laboratory for TSE; the Morphopatology Department of the Institute for Diagnosis and Animal Health.

In the frame of passive and active surveillance for BSE on large ruminants,1720 histological exams, 510166 rapid tests, 2765 immunoblotting tests and 286 imunohistochemical tests were performed and after applying immunoblotting and imunohistochemical tests being confirmed 2 atypical cases of BSE.

The differentiation tests were performed by the Eu-RL-TSE of the Animal and Plant Health Agency (APHA) Weybridge, United Kingdom, the atypical L-type BSE being diagnosed at the 2 cases.

KEYWORDS. atypical form, BSE, confirmation tests, L-type, rapid tests

P-160: Rapid tests might overlook bovine spongiform encephalopathy infection in goats

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Scrapie disease of sheep and goats has been endemic in Europe for more than 200 years, but has never been convincingly associated with any form of human TSE disease, though recent data based on experimental transmission of scrapie to humanized mice or non-human primates have re-opened this issue. On the other hand, the epidemic of bovine spongiform encephalopathy (BSE) in the UK and other European cattle population has been unequivocally linked to the appearance of variant Creutzfeldt-Jakob disease. A specific investigation on the suitability of EU approved rapid methods for the detection of TSE diseases in goats was never performed. The aim of this study was to compare the performance of IDEXX HerdChek® BSE-scrapie (IDEXX), Bio-Rad® TeSeE SAP (BIORAD-SAP) and Bio-Rad® TeSeE Sheep/Goat (BIORAD-SG) tests on brain samples from goats with natural scrapie, experimental scrapie or BSE. Thirty-one of these samples were sourced from goats with natural scrapie from 7 different EU countries: 7 from clinically affected goats and 24 from clinically healthy animals. Other samples (n = 32) from goats with experimentally induced scrapie or BSE were provided by the CVI, FLI, Roslin, INRA, and CEA institutes. Different PRNP genotypes characterized the positive samples. Overall, the 3 rapid tests showed 100% specificity and over 80% sensitivity, being the IDEXX significantly more sensitive than the Bio-Rad tests. All tests recognized 100% of samples from goats with clinical scrapie, either experimentally or naturally infected. In contrast, the sensitivity was lower in goats with pre-clinical scrapie, where IDEXX missed about 7% of samples, BIORAD-SAP 14%, and BIORAD-SG up to 24%. Finally, IDEXX picked up all the samples from clinical BSE-infected goats, while the other 2 rapid tests missed the 15% (BIORAD-SG) to the 25% (BIORAD-SAP). Overall, 2 concerns come from these results: i) that pre-clinical scrapie infections may be missed by EU surveillance, with sensitivity of detection strongly dependent on the rapid test used, and most importantly ii) that a significant proportion of clinical BSE infections may be overlooked when using BIORAD rapid tests. Assuming that the same sensitivity on pre-clinical goats would also occur in BSE-infected goats, our data show that only the IDEXX test would be possibly suitable for detecting eventual preclinical field case of BSE infection in goats, though with a disappointing 7% failure. Our results cast some concerns in relation to the reliability of current figures on BSE infections in goats deriving from EU surveillance.

P-162: Local and traditional knowledge in monitoring of chronic wasting disease and wildlife health in Western Canada

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The health of wildlife and the health of Indigenous peoples are strongly interconnected in many countries including Canada. In western Canada, Indigenous peoples depend on deer, elk, moose and caribou for food and as part of their livelihood and culture. For this reason, we know that wildlife diseases including CWD can negatively affect traditional diets and also the health and wellbeing of Indigenous communities. However, communities are not simply vulnerable - many harvesters are knowledgeable (i.e. hold traditional knowledge) and are willing to participate in wildlife monitoring to ensure the long term health of both the animals and their communities. Monitoring of wildlife health is not a new idea to many harvesters but is part of their traditional way of life. For generations they have systematically tracked such indicators as animal condition (e.g., skinny/fat liver) to ensure a healthy diet. Although different methods and language are often used, traditional monitoring systems are similar to those used by scientists. Our research will involve working closely with First Nations organizations, communities and harvesters to understand more about traditional systems of monitoring and how they might be important to addressing the spread and effects of CWD.

P-163: Active vaccination against chronic wasting disease using multimeric rec-PrP: A promising approach to contain CWD

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Chronic wasting disease (CWD) is the sole prion disease which occurs in free ranging and captive herds. It expands in North America and the potential for transmission into humans cannot be excluded yet. Effective measures for controlling CWD are therefore crucial. Our hypothesis here is the feasibility to interfere with peripheral prion infection and prion shedding by inducing autoantibodies against PrP^c by active vaccination. Our rationale is to overcome self-tolerance against PrP by using a β sheeted multimeric recombinant PrP as immunogen. As our previous experimental data in wild type mice have shown, this approach induces robust humoral and cellular responses against PrP^c. Having a solid proof-of-concept for our strategy in wild-type mice we are now focusing on active vaccination against CWD. We have expressed in*E.coli* purified and refolded 4 immunogens, cervid and murine PrP in monomeric and dimeric form. As a delivery strategy allowing oral vaccination, we encapsulated recombinant PrPs with adjuvants into PLGA microspheres. Then by testing immunogenicity in sera of vaccinated transgenic deer PrP mice, we found that all 4 immunogens, monomeric and dimeric, could effectively overcome the selftolerance against the prion protein as shown by very high antibody titers (dilutions up to 1: 30,000 were still reactive in end point ELISA titration in some groups). Additionally, post-immune sera were reactive against rec PrP in western blotting. Using the epitope mapping we determined the key epitopes responsible for the immunogens reactivity.

Protection against CWD will be tested on ScN2a cells, CWD-infected RK13 cells and by challenging the immunized mice with prions. Our long term goal is to implement a translational research program for developing a wildlife vaccine which is able to reduce levels of morbidity and mortality of CWD.

P-164: Adsorption of Soluble prions by metals is limited by plasma

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Background: The demonstration that variant Creutzfeldt-Jakob disease can be transmitted through blood and plasma derivatives pushed ahead the development of measures to increase the safety of transfusions. Although manufacturing steps used in the production of plasma derivatives already have the capacity to decrease prion infectivity, the reduction observed is not sufficient to eliminate the risk of iatrogenic transmission, calling for more specific and efficient methods.

In spite of the great efforts spent in this direction, no tools have still been made available that meet the required level of protection.

We here present the results of a study (supported by the Alliance BioSecure Foundation) designed to evaluate the capacity of different metals to adsorb and remove prions from biological fluids.

Study Design and Methods: Aggregated and soluble brain prions dispersed in buffered saline were exposed to metal powders (nickel, steel, molybdenum) under different conditions (time, prion/metal ratio, temperature, stirring speed) to identify the combination of parameters that yields the highest prion removal. Residual prions were assayed by western blot (aggregated prions) and animal bioassay (both aggregated and soluble prions). The combination of conditions that performed best was then applied on infected plasma (taken from affected hamsters or spiked with soluble brain prions) and the infectivity was measured by animal bioassay.

Results: As a first result we confirmed the capacity of metal powders to sequester aggregated prion infectivity. Secondly, we identified a definite set of optimal conditions that produced a relative removal of infectivity higher than one order of magnitude (about 1.3 log), corresponding to a binding capacity of 4.28 log LD50 of soluble prions per gram of nickel (or molybdenum). Thirdly, we found that the binding capacity of soluble prions by nickel was completely abrogated in the presence of plasma, regardless of the nature (endogenous or exogenous) of infectivity.

Conclusion: Our results convincingly demonstrate that metals are able to remove both aggregated and soluble brain prions from complex biological solutions, however, the modest size of the removal factor and the complete inhibition observed in the presence of plasma suggest that further investigation and optimization are necessary to disclose the potential of this tool for the safety of plasma products.

The finding that the prion binding capacity of metals is blocked in the presence of raw plasma may have some relevance when evaluating the risk of infectivity carryover (and batch cross-contamination) by prions adsorbed onto metal surfaces of plasma derivatives production pipeline.

P-165: CJD incidents in Japan

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CJD incidents is announced when the CJD high-risk surgeries, such as neurological surgeries or the others with the penetration of the dura matter, are performed in the CJD subjects, and the surgical tools used are sterilized inappropriately afterwards, and when those instruments are used in the subsequent patients. These subsequent patients are called 'possible CJD risk holders', and should be followed up annually for 10 y. The number of follow up 'possible CJD risk holders' are defined according to the numbers of sets of neurosurgical tools used in that CJD incident, and this number is defined after the on-site investigation by the CJD incident committee. One of the difficulties of its understanding lies in the fact that the period of that particular incident could be prior 1-2 y to the clinical manifestation of CJD, and it is impossible in the clinical practice to predict if one will future be involved in CJD without the presence of clinical signs of CJD, especially with the ones without genetic backgrounds, such as sporadic CJDs. Standard precaution of the surgical tools sterilization to aim at the CJD deactivation is strictly required that will be used in the CJD high risk surgeries, such as neurological surgery.

We have been following up 15 cases of CJD incidents as of now in Japan. Those CJD incidents are found and pointed out in the process of the Japan CJD surveillance. Many of those incidents are regular neurosurgical practices, such as irrigation of chronic subdural hematomas or the shunt surgeries, rather than the biopsies for the non intracranial-space occupying lesions. To minimize these CJD incidents in Japan, we have defined the Japan CJD guidelines 2008 which is focusing on the sterilization, and we have established CJD incident committee in 2011. As a result, although the annual number of the CJD incident occurrence is not decreasing, the number of the preventable incident, the incident after the CJD clinical manifestation, is decreased.

There is no single case of secondary transmission of CJD from the possible CJD risk holders in our follow up.

P-167: Sorting of prion protein and PrP^{Sc} accumulation

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A fundamental event in the pathogenesis of prion disease is the conversion of host cellular prion protein (PrP^{c}) into the abnormally folded isoform (PrP^{Sc}) that abundantly accumulates in the prion-infected brain with the progression of the disease. Here we demonstrate our recent findings on a molecular mechanism for sorting of PrP to late endosomal/lysosomal compartments, which is related to excessive accumulation of PrP^{Sc}. We identified Sortilin as a novel sorting receptor for PrP. Sortilin is a member of VPS10P sorting receptor family which contains 5 members, Sortilin, SorCS1, SorCS2, SorCS3, and SorLA. They bind to cargo proteins

through their N-terminal luminal/extracellular VPS10P domains and transport cargo proteins from donor to target organelle in post-Golgi membrane traffic pathways. Their C-terminal cytoplasmic domains contribute to the formation of transport carriers. We show that Sortilin functions as the sorting receptor for PrP^{c} to internalize and transport it from cell surface to late endosome/lysosome. In addition, it can interact with both PrP^{c} and PrP^{Sc} and its dysfunction in uninfected and prion-infected cells increases PrP^{c} and PrP^{Sc}, respectively. Notably, prion-infection reduces the expression of Sortilin in cultured cells and mouse brains. These findings suggest that the Sortilin-dependent PrP sorting to the lysosomal degradation pathway plays an important role in PrP^{Sc} degradation and its disorder by prion-infection leads to the abundant accumulation of PrP^{Sc} in prion disease.

P-168: Three-dimensional cultures of murine neurones demonstrate prion-induced plaque pathology and cell death

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The ability to grow neuronal cultures in 3-dimensional space offers the potential to model neurodegenerative disease-associated changes such as plaque formation and spread; changes that are rarely evident in 2-dimensional neuronal culture systems. We have previously found that, when differentiated in 2-dimensional culture, neurones and astrocytes from murine neural stem cells demonstrate toxic changes within 7 d of exposure to prions. To further this work we have developed a new method for generating neuronal cultures from adult murine neural stem cells. Using this method, mature 3-dimensional cultures are generated within 10 days, remain healthy beyond a month and demonstrate a higher neuronal content than those differentiated on a 2-dimensional matrix. Neuronal content could additionally be influenced by culture size and growth conditions. Furthermore, 3-dimensional neuronal cultures were able to be infected with our M1000 prion strain. Disease was induced within these cultures by a single 72-hour exposure to M1000 prions from infected mouse brain homogenates in constantly agitated culture media, with normal, uninfected brain homogenates used to treat control cultures. Cultures were imaged following incubation with fluorescent probes that detect caspase (death effector protein) activation weekly for 3 weeks following infection. The cultures showed progressive infection-associated activation of cell death pathways. Examination of the cultures displaying heightened caspase activation showed death was accompanied by development of thioflavin-T positive deposits of the prion protein replicating plaque formation. Further utility of these cultures was verified by incubation with a novel in house compound designed as an anti-prion therapeutic, which demonstrated the ability to prevent plaque formation. Our data make evident that 3-dimensional neuronal cultures can be used to recapitulate prion-disease pathology and can be applied to discovery of novel therapeutics for prevention of plaque formation or disassembly of existing plaques.

P-169: Building a program for community-based monitoring of wildlife health - Lessons for surveillance of chronic wasting disease in moose and deer in Western Canada

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The inclusion of Traditional Knowledge in the monitoring and management of wildlife health is being increasingly recognized as best practice by biologists, governments and other

scientists. Indeed, the systematic observations and related data gathered by active harvesters can be the best (and sometimes only) available "data" about wildlife health (including the spread of wildlife diseases), particularly in regions where other capacities and resources for monitoring are limited. Although synergistic with science in many respects, Traditional Knowledge holders can also speak to the socio-economic, cultural and spiritual significance of changes occurring as results of the spread of wildlife disease. The presentation will highlight results of a study funded by the Alberta Prion Research Institute (Canada). The research collaboration between Treaty 8 First Nations of Alberta and the University of Alberta is contributing to knowledge about, and capacities for, community-based monitoring of wildlife health through the documentation of oral histories, cultural and ecological indicators and traditional monitoring practices.

P-170: CJD International Support Alliance (CJDISA) - The voice, the face, the human story behind this horrific disease

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The CJD International Support Alliance (CJDISA) was formed by a group of grassroots nonprofit organizations that work together as an international coalition on behalf of patients and families affected by prion disease and those at risk the world over. CJDISA was founded to fill the gap that exists on an international level and to assure excellence in the service to individuals affected/at risk of prion disease, their families, and caregivers. The participating organizations are dedicated to work together in meeting the educational, social, emotional, spiritual and practical needs of those they represent. Under the CJDISA umbrella, these organizations collaborate on educational initiatives, information dissemination, resource allocation, program design and implementation, and advocacy.

The CJDISA was founded on the belief that by raising awareness of prion disease and educating healthcare professionals and the public at large, we could:

Help remove the stigma surrounding CJD and other prion diseases

Promote research activities around early detection, prevention, treatment opportunities, improved quality of life and ultimately a cure

Increase proper utilization of resources

Promote continued access to care for those in active disease or those at risk for prion disease

Assist in ensuring safe blood and food supply.

Our work is guided by 31 prion researchers and professionals, dedicated to support families affected by CJD and other prion disease and form the 'Friends and Advisors Group' of the CJDISA. These individuals offer their expert guidance on various programs and services established by the CJDISA, provide lay interpretation of research articles so that families can understand how that research may or may not apply to them, and they act as knowledgeable resources for countries that don't have a CJD support organization yet have patients and families in need of assistance.

A priority for the CJDISA is to assist counties in the establishment of CJD Support Organizations wherever and whenever possible. Having support organizations in each country is critical in order to provide timely and accurate assistance to patients and families in their native country. Healthcare, infection prevention, and embalming/burial regulations vary between countries. During time of great stress and sadness families deserve the most knowledgeable resources available to them, guiding them through the tragedies of prion disease.

Member organizations benefit from the involvement of people directly impacted by prion

disease. We are the voice, the face, the human story behind this horrific disease representing those impacted by prion disease on a global level.

P-171: Presymptomatic genetic testing for genetic prion disease: What should we consider and how should we deal with it?

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Genetic Creutzfeldt-Jakob disease, Gerstmann-Straussler-Scheinker syndrome, and Fatal Familial Insomnia are known as a genetic form of prion disease, which is an autosomal dominant hereditary condition. According to the Japanese prion disease surveillance program data, genetic cases account for around 15% of total prion disease patients. Recently, we have seen some unaffected family members of genetic prion disease patients, who are so-called "at-risk individuals." There are some at-risk individuals who are seeking presymptomatic, predictive genetic testing to get to know their genetic status, so that they can think about their future life plan and/or family planning, or sometimes they just want to know. In the future, it will potentially happen that at-risk individuals will be good candidates for the prevention drug clinical trials. And then, they have to undergo presymptomatic genetic testing before the drug use. Thus, it is critical to consider some important points beforehand to have well-established system of presymptomatic genetic testing for genetic prion disease.

As genetic prion disease is a condition which does not have any prevention or cure, in order to conduct presymptomatic genetic testing, like Huntington disease, there are some things that should be considered and discussed prior to the testing. To specify what we should consider, we reviewed related guidelines, such as genetic testing for adult-onset condition guidelines of National Society of Genetic Counselors (NSGC, USA), American College of Medical Genetics (ACMG), as well as Huntington disease presymptomatic genetic testing guidelines of World Federation of Neurology and International Huntington Association, and familial Alzheimer disease presymptomatic testing guidelines of NSGC and ACMG.

The themes we extracted from those guidelines that are needed to pay attention to are 1) provision of accurate up-to-date, and enough information, 2) consideration of background psychiatric conditions and history, 3) psychological preparation and readiness, 4) autonomous decision making process, 5) privacy, 6) family issues, 7) genetic discrimination, 8) ownership of the test result, 9) duty of the medical center and testing laboratory, 10) genetic counseling, 11) supportive member(s) of the at-risk individual's family, 12) how to deliver the result, 13) follow-up, 14) other options. In this presentation, the details of these themes will be discussed.