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Prion 2015 Oral Abstracts

Propagation

O.01: Transgenic mice expressing human wild-type α-synuclein develop neuropathology after inoculation with brain homogenates from patients with multiple system atrophy or aged subjects without neurological disorder

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Multiple system atrophy (MSA) and Parkinson's disease are synucleinopathies that are defined by the presence of aggregated and hyperphosphorylated α-synuclein (α-syn) within cells of the central nervous system (CNS).

Recent findings suggest that pathological α-syn may spread prion-like within the nervous system. We investigated prion-like propagation of pathological α-syn in Tg(SNCA)1Nbm/J mice that do not express mouse but low levels of human wt α-syn and do not naturally develop any pathology or neurodegenerative disease.

We inoculated brain homogenate from 2 patients with MSA, from 2 aged control subjects without neurological disorder, or saline intrastriatally into Tg(SNCA)1Nbm/J mice. Challenged mice were sacrificed at 90, 180, and 270 d post inoculation and were analyzed biochemically and immunohistochemically for pathological α-syn.

Brain homogenates from MSA or aged control subjects but not saline triggered progressive accumulation of aggregated α-syn in neurons of inoculated mice. Aggregates of α-syn were hyperphosphorylated and co-stained for p62 that targets proteins for degradation. Aggregates of pathological α-syn were first observable in the ipsilateral brain hemisphere and over time in the contralateral hemisphere and in more rostral and caudal areas.

Our findings show that brain homogenate from MSA patients but not saline induces pathological changes in the CNS of Tg(SNCA) 1Nbm/J mice. Our data support that pathological α-syn may propagate prion-like along neuronal networks. Furthermore, human wt α-syn supports propagation of pathological α-syn. Intriguingly, brain homogenate from aged control subjects without neurological disorder equally induced synucleinopathy in brains of Tg(SNCA)1Nbm/J mice suggesting that aged human brains can contain pathological α-syn.

O.02: Experimental transmissibility of mutant SOD1 motor neuron disease

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By unknown mechanisms, the symptoms of amyotrophic lateral sclerosis (ALS) seem to spread along anatomical pathways to engulf the motor nervous system. The rate at which symptoms spread is one of the primary drivers of disease progression. One mechanism by which ALS symptoms could spread is by a prion-like propagation of a toxic misfolded protein from cell to cell along anatomic pathways. Proteins that can transmit toxic conformations between

cells often can also experimentally transmit disease between individual organisms. To survey the ease with which motor neuron disease (MND) can be transmitted, we injected spinal cord homogenates prepared from paralyzed mice expressing mutant superoxide dismutase 1 (SOD1-G93A and G37R) into the spinal cords of genetically vulnerable SOD1 transgenic mice. From the various models we tested, one emerged as showing high vulnerability. Tissue homogenates from paralyzed G93A expressing mice induced MND in 6 of 10 mice expressing low levels of G85R-SOD1 fused to yellow fluorescent protein (G85R-YFP mice) by 3–11 months, and produced widespread spinal inclusion pathology. Importantly, second passage of homogenates from G93A→G85R-YFP mice back into newborn G85R-YFP mice, induced disease in 4 of 4 mice by 3 months of age. Homogenates from paralyzed mice expressing the G37R variant were among those that transmitted poorly regardless of the strain of recipient transgenic animal injected, a finding suggestive of strain-like properties that manifest as differing abilities to transmit MND. Together, our data provide a working model for MND transmission to study the pathogenesis of ALS.

O.03: Role of brain interstitial fluid flow in very early generation and spread of PrPres after microinjection of prion infectivity in C57BL mouse brain

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Prion infectivity typically spreads along neurons following the paths of neuron circuitry within the CNS; however, less is known about the process of initial brain infection. Here we studied spread of disease-associated PrPres in brain of C57BL mice from 30 minutes to 40 d after microinjection (0.5 μl) of scrapie infectivity into the striatum. After microinjection of 22L scrapie, PrPres was visible in the needle track and around nearby blood vessels at 30 min in both C57BL and Prnp-null (KO) control mice. This rapid initial spread to vessels appeared to be via brain interstitial fluid (ISF) flow in perivascular and periaxonal regions. At 3 dpi in KO mice, inoculated PrPres was mostly gone. Remarkably, at 3 and 7 dpi in C57BL mice, generation of new PrPres was detectable by immunohistochemistry, immunoblot and RT-QUIC assay. Again, PrPres was associated with perivascular sites of ISF drainage, and PrPres associated mainly with perivascular astroglia, and only minimally with neurons, microglia and oligodendroglia. By 20–40 dpi, PrPres had spread to ipsilateral thalamus and cerebral cortex at locations 1.5–2.5 mm distant from the injection site. Both the ipsilateral PrPres distribution and the rapid transit time suggested that spread from striatum was via neuronal circuity. Thus early 22L scrapie spread involved both neurons and ISF flow, however PrPres mainly accumulated in astroglia. In experiments using scrapie strain ME7, similar spreading mechanisms were observed, but PrPres accumulated primarily in association with neuronal cell bodies and neuropil, which was not seen with strain 22L.

O.04: A single coding polymorphism in the *PRNP* gene significantly alters the transmission dynamics of blood-borne prions

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Sheep experimentally infected with bovine spongiform encephalopathy (BSE) have provided a useful model in which to study the risks of transmission of prion disease by transfusion of blood components. We have previously shown that several components relevant to human clinical practice (red cell concentrate, platelets, plasma) are infectious, and that leucodepletion of these components does not completely prevent disease transmission.

Further analysis of the data has identified factors which distinguish donor sheep that transmitted infection *via* blood components ("transmitters"), from those that did not ("non-transmitters"). The codon 141 polymorphism of the sheep *PRNP* gene (L → F) was strongly associated with the probability of transmitting infection, with the majority of transmitters having a 141LL genotype, while the majority of non-transmitters were 141LF. Since codon 141 genotype has also been associated with variation in incubation period, this finding may reflect the stage of the incubation period reached by the donor at the time when blood was collected, because titres of infectivity in blood tend to increase as animals progress toward the clinical phase of infection. Another factor that appears to differ between transmitting and non-transmitting donors is the extent of PrP^d deposition in lymphoid tissues, with transmitters having a higher proportion of positive lymphoid tissues than non-transmitters, regardless of their codon 141 genotype. This suggests that a single nucleotide polymorphism of *PRNP*, and/or the extent of prion replication in lymphoid tissues, may influence titres of blood-borne infectivity and thus the risk of disease transmission by transfusion of blood components.

O.05: Transmission of prions to primates after extended silent incubation periods: Implications for BSE and scrapie risk assessment in human populations

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Prion diseases (PD) are the unique neurodegenerative proteinopathies reputed to be transmissible under field conditions since decades. The transmission of Bovine Spongiform Encephalopathy (BSE) to humans evidenced that an animal PD might be zoonotic under appropriate conditions. Contrarily, in the absence of obvious (epidemiological or experimental) elements supporting a transmission or genetic predispositions, PD, like the other proteinopathies, are reputed to occur spontaneously (atpical animal prion strains, sporadic CJD summing 80% of human prion cases).

Non-human primate models provided the first evidences supporting the transmissibiity of human prion strains and the zoonotic potential of BSE. Among them, cynomolgus macaques brought major information for BSE risk assessment for human health (Chen, 2014), according to their phylogenetic proximity to humans and extended lifetime. We used this model to assess the zoonotic potential of other animal PD from bovine, ovine and cervid origins even after very long silent incubation periods.

We recently observed the direct transmission of a natural classical scrapie isolate to macaque after a 10-year silent incubation period, with features similar to some reported for human cases of sporadic CJD, albeit requiring fourfold longe incubation than BSE. Scrapie, as recently evoked in humanized mice (Cassard, 2014), is the third potentially zoonotic PD (with BSE and L-type BSE), thus questioning the origin of human sporadic cases. We will present an updated panorama of our different transmission studies and discuss the implications of such extended incubation periods on risk assessment of animal PD for human health.

O.06: Prion properties of the Alzheimer's disease associated proteins in the yeast model

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Amyloid formation is implicated in various human diseases, and many amyloids are

suspected to possess transmissible (prion) properties. However, molecular mechanisms of amyloid formation and propagation are difficult to investigate in vivo due to complexity of the human organism. We have established a yeast model for studying the prion properties of mammalian (including human) proteins. Our model employs chimeric constructs, containing the mammalian amyloidogenic proteins (or domains) fused to various fragments of the yeast prion protein Sup35. Phenotypic and biochemical detection assays, previously developed for the Sup35 prion, enable us to detect prion nucleation and propagation by mammalian proteins. By using this approach, we have investigated prion properties of Abeta and tau proteins. Oligomerization and aggregation of Abeta and tau is known to be associated with Alzheimer's disease in humans. We have shown that both proteins confer prion characteristics to the chimeric constructs in yeast. Effects of known pro-aggregation and anti-aggregation mutations in these proteins on prion formation in yeast generally correspond to their effects on the disease development in humans. For example, the D23N substitution in Abeta increases prion nucleation in the yeast system. New mutations with predicted effects on amyloidogenesis are being generated and tested. Formation of different prion "strains" by chimeric proteins has been detected in yeast. Assays for studying interactions between different amyloidogenic proteins in the yeast cell have been developed. Overall, yeast model enables us to perform genetic dissection of molecular processes leading to the initiation of Alzheimer's disease.

Topics in Animal Prions

O.07: 2-aminothiazole treatment of chronic wasting disease in transgenic mice expressing elk PrP

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Treatment with the 2-aminothiazole IND24 extended the survival of wild-type mice infected with mouse-passaged RML scrapie prions, but also resulted in the emergence of a drug-resistant prion strain. IND24 treatment was also efficacious against Tg mice expressing ElkPrP and infected with chronic wasting disease (CWD) prions, but the impact of treatment on the properties of CWD prions was not determined. Here, we assessed whether IND24 treatment extended the survival of additional natural isolates in Tg mice infected with sheep scrapie or CWD prions using 2 isolates for each disease. Multiple IND24 treatment regimens doubled the incubation times for CWD-infected mice, but IND24 treatment had no effect on the survival of the ovine scrapie-infected mice. Biochemical, neuropathologic, and cell culture analyses were used to characterize the prion strain properties following treatment, and indicated that the CWD prions were not altered by IND24 treatment regardless of survival extension. A second passage in the absence of treatment reproduced the original survival time, and cells infected with prions from animals that were treated with IND24 were as susceptible to IND24 treatment as CWD prions that were never before exposed to IND24. These results suggest that IND24 may be a viable candidate for treating CWD in infected captive cervid populations, and raise questions about why some strains acquire resistance upon treatment whereas others do not.

O.08: H-type bovine spongiform encephalopathy associated with E211K prion protein polymorphism: Clinical and pathologic features in wild-type and E211K cattle following intracranial inoculation

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In 2006 an H-type bovine spongiform encephalopathy (BSE) case was reported in an animal with an unusual polymorphism (E211K) in the prion protein gene. Although the prevalence of this polymorphism is low, cattle carrying the K211 allele are predisposed to rapid onset of H-type BSE when exposed. The purpose of this study was to investigate the phenotype of this BSE strain in wild-type (E211E) and E211K heterozygous cattle.

One calf carrying the wild-type allele and one E211K calf were inoculated intracranially with H-type BSE brain homogenate from the US 2006 case that also carried one K211 allelle. In addition, one wild-type calf and one E211K calf were inoculated intracranially with brain homogenate from a US 2003 classical BSE case. All animals succumbed to clinical disease. Survival times for E211K H-type BSE inoculated catttle (10 and 18 months) were shorter than the classical BSE inoculated cattle (both 26 months). Significant changes in retinal function were observed in H-type BSE challenged cattle only. Animals challenged with the same inoculum showed similar severity and neuroanatomical distribution of vacuolation and disease-associated prion protein deposition in the brain, though differences in neuropathology were observed between E211K H-type BSE and classical BSE inoculated animals. Western blot results for brain tissue from challenged animals were consistent with the inoculum strains.

This study demonstrates that the phenotype of E211K H-type BSE remains stable when transmitted to cattle without the E211K polymorphism, and exhibits a number of features that differ from classical BSE in both wild-type and E211K cattle.

Mechanisms of Disease

O.09: Mutant prion proteins related to genetic prion diseases impair intracellular trafficking

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Fatal familial insomnia (FFI), genetic Creutzfeldt-Jakob disease (gCJD) and Gerstmann-Sträussler-Scheinker (GSS) syndrome are neurodegenerative disorders linked to mutations in the prion protein (PrP) gene. The mechanism of neurotoxicity of mutant PrP is not clear, but misfolding and intracellular accumulation may contribute to the pathogenic process. We previously found that mouse (mo) PrP D177N/M128, homologous to the D178N/M129 mutation associated with FFI, accumulates in the Golgi of neuronal cells, impairing post-Golgi trafficking.[1] To further characterize the trafficking defect induced by the FFI mutation, and test whether other mutants had similar effects, we analyzed the efficiency of secretory transport in cells expressing moPrP D177N/M128, D177N/V128 (homologous to the D178N/V129 mutation linked to gCJD), and PG14, a 9-octapeptide repeat insertion associated with GSS. We used transfected HeLa cells, embryonic fibroblasts and primary neurons from transgenic mice, as well as human fibroblasts from carriers of the FFI mutation. In all cell models mutant PrPs showed abnormal intracellular localizations, accumulating in different compartments of the secretory pathway. To test the efficiency of the membrane

trafficking system, we monitored the intracellular transport of the temperature-sensitive vesicular stomatite virus glycoprotein (VSVG), a well-established cargo reporter. We observed marked alterations in secretory transport, with VSVG accumulating mainly in the Golgi complex. Our results indicate that different pathogenic mutations share the property of impairing intracellular trafficking. This suggests that defective intracellular transport may be a general mechanism of neurotoxicity in genetic prion diseases.

1. Massignan T, Biasini E, Lauranzano E, Veglianese P, Pignataro M, Fioriti L, Harris DA, Salmona M, Chiesa R, Bonetto V. Mutant prion protein expression is associated with an alteration of the Rab GDP dissociation inhibitor alpha (GDI)/Rab11 pathway. Mol Cell Proteomics 2010; 9(4):611–622; PMID: 19996123; http://dx.doi.org/10.1074/mcp.M900271-MCP200

O.10: The sheddase ADAM10 significantly impacts on prion disease

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Proteolytic processing of key proteins, be it deleterious or protective, is relevant in many neurodegenerative proteinopathies such as Alzheimer's or prion disease. With regard to the latter, while α-cleavage in the middle of the cellular prion protein (PrP^C) has been shown to impair misfolding into the pathogenic isoform (PrP^{Sc}) and thus to be protective against prion disease, the role of another physiological cleavage (i.e., shedding in close proximity to the GPI-anchor of PrP^C) remained largely unknown. We and others have identified ADAM10 as the physiologically relevant sheddase of PrP^C regulating its membrane homeostasis.

Using a novel mouse model, we show that depletion of ADAM10 in forebrain neurons leads to posttranslational increase of PrP^C levels. When infected with prions, these mice present with drastically shortened incubation times, increased PrP^{Sc} formation and upregulation of calpain. Our spatiotemporal analyses also suggest that absence of shedding impairs spread of prion pathology within the brain. Moreover, our mouse model provides some interesting insights into central issues discussed in the prion field, such as (i) an inhibitory effect of anchorless PrP versions on the conversion process, (ii) mechanisms of prion-associated neurotoxicity, and (iii) a likely disparity between PrP^{Sc} amounts and prion infectivity.

Taken together, ADAM10-mediated shedding seems to have a dual role in prion diseases thus emphasizing the relevance of proteolytic processing in these conditions. Given the suggested role of PrP^C as a receptor for toxic protein oligomers in more common proteinopathies our findings might impact on these devastating conditions as well.

Structure/Function

O.11: Structural determinants of phenotypic diversity and replication rate of human prions

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The infectious pathogen responsible for prion diseases is the misfolded, aggregated form of the prion protein, PrP^{Sc}. In contrast to recent progress in studies of laboratory rodent-adapted prions, current understanding of the

molecular basis of human prion diseases and, especially, their vast phenotypic diversity is very limited. Here, we have purified proteinase resistant PrP^{Sc} aggregates from two major phenotypes of sporadic Creutzfeldt-Jakob disease (sCJD), determined their conformational stability and replication tempo in vitro, as well as characterized structural organization using recently emerged approaches based on hydrogen/deuterium (H/D) exchange coupled with mass spectrometry. Our data clearly demonstrate that these phenotypically distant prions differ in a major way with regard to their structural organization, both at the level of the polypeptide backbone (as indicated by backbone amide H/D exchange data) as well as the quaternary packing arrangements (as indicated by H/D exchange kinetics for histidine side chains). Furthermore, these data indicate that, in contrast to previous observations on yeast and some murine prion strains, the replication rate of sCJD prions is primarily determined not by conformational stability but by specific structural features that control the growth rate of prion protein aggregates.

O.12: Scrapie-specific C-terminal antibody reveals conformational differences between prion strains

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Misfolding of the cellular form of prion protein (PrP^{C}) into the pathological PrP (PrP^{Sc}) in the brain can lead to transmissible spongiform encephalopathies or prion diseases in humans and animals. PrP^{C} is an alpha-helix-rich monomer, while PrP^{Sc} forms beta-sheet-rich oligomers and amyloid fibrils. Prion strains have been classified based on different incubation times, neuropathological lesions and biochemical characteristics. However, the detailed structure of PrP^{Sc} and conformational differences between strains are poorly understood. In this study, we used antibodies to different epitopes to probe the structures of PrP^{Sc} isolated from brains of mice with either the Chandler or 22L strains or hamsters with 263K scrapie. Epitope mapping of PrP^{Sc} was performed under native or guanidine-denatured conditions by indirect-ELISA. Our results showed that only a small subset of antibodies recognized epitopes in the native structure of PrP^{Sc}. One of those antibodies, with a conformationally sensitive C-terminal epitope, had strongly differing reactivities to the native Chandler and 22L strains of PrP^{Sc} despite the fact that these murine prions share the same PrP primary structure. Although it has long been apparent that prion strains can differ conformationally near the N-termini of molecules forming the proteinase-K resistant core of PrP^{Sc}, our results show evidence for stain-dependent conformational differences near the C-terminal as well.

O.13: Misfolded wild-type SOD1 induced by pathological FUS or TDP-43 transmits intercellularly and is propagation-competent

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Clinically indistinguishable cases of amyotrophic lateral sclerosis (ALS) can be caused by either inheritable mutation in the genes encoding SOD1, TDP-43, FUS, among others, or can occur sporadically. Misfolded SOD1 has been detected in both familial and sporadic ALS patients, despite SOD1 mutations accounting for only ~2% of total cases. We previously reported that pathological FUS or TDP-43 kindles misfolding of human wild-type (wt) SOD1 in living cells. Here, we use human

cell cultures and mouse primary neural cultures expressing human wtSOD1, to establish that FUS or TDP-43-induced misfolded SOD1 can traverse between cells through the incubation of untransfected cells with conditioned media, triggering conversion of endogenous SOD1 in a prion-like fashion. This intercellular spread is arrested by pre-incubation of the conditioned media with misfolded SOD1-specific antibodies, demonstrating their therapeutic potential. We find that recipient cells pre-treated with SOD1-siRNA do not contain misfolded SOD1, indicating that endogenous SOD1 is required as substrate for active conversion. Our data also shows that conditioned media obtained from mutant TDP-43 and FUS transfected, or wild-type TDP-43 over-expressing, cells is cytotoxic to the recipient cells. Furthermore, transfection of TDP-43 into cells triggers its cleavage, mis-localization and hyperphosphorylation; these properties are not observed in untransfected cells incubated with conditioned media from pathological TDP-43 transfected cells, further confirming that the transmission of SOD1 misfolding occurs independently of TDP-43.

O.14: The architecture of recombinant prions is similar to that of brain-derived prions: Insights from limited proteolysis

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

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

Limited proteolysis of a variety of mouse and bank vole $recPrP^{Sc}$ species, prepared under slightly different conditions, yields the same N-terminally truncated PK-resistant fragments seen in brain-derived PrP^{Sc}, indicative of an overall similar architecture of both prion types. However, lower resistance to PK and a comparatively higher abundance of smaller fragments with respect to the "canonic" ~90–230 PK-resistant core, suggests higher flexibility and nuances in threading for $recPrP^{Sc}$. Furthermore, doubly N- and C-terminally truncated fragments, in particular ~90–152, are often detected; similar fragments are characteristic of atypical strains of brain-derived PrP^{Sc}.

Ongoing comparison of specific digestion patterns (relative abundances of individual fragments) and incubation times will allow extracting conclusions on relationships between structure and biological properties of different $recPrP^{Sc}$ species. Recombinant PrP^{Sc} offers exciting opportunities for structural studies not possible to date with brain-derived PrP^{Sc}.

O.15: In a mammalian model of epithelial-to-mesenchymal transition the prion protein mediates β-catenin-dependent transcriptional activation of a key EMT regulator

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The physiological function of the prion protein (PrP) has remained elusive despite its widely recognized role in neurodegenerative diseases and sustained efforts to understand its molecular biology. On the basis of its evolutionary relationship to ZIP zinc transporters and the characteristics of a gastrulation arrest phenotype in a PrP-deficient zebrafish model, we considered that PrP may contribute to the morphogenetic reprogramming of cells underlying epithelial-to-mesenchymal (EMT) transitions. We now report that consistent with this hypothesis, PrP levels can be observed to increase more than 5-fold during EMT, and its CRISPR-Cas9-mediated knockout interferes with EMT in NMuMG cells, a widely used mouse model for studying this cellular program. Subsequent endeavors to dissect the molecular underpinnings of this phenotype revealed that PrP-deficient cells fail to execute an essential step during EMT. Surprisingly, this impairment was caused by a failure of PrP-deficient cells to activate transcription of a critical EMT mediator. A subsequent comparative global proteome analysis of wild-type and PrP-deficient NMuMG cells pointed toward β-catenin as a transcriptional regulator that contributes to this deficiency of PrP-knockout cells. Indeed, pharmacological blockade or siRNA-based knockdown of β-catenin mimicked PrP-deficiency. By placing PrP in a signaling pathway that is essential for EMT in NMuMG cells, our study introduces an easily accessible model for studying signaling downstream of PrP, and provides a fresh angle for understanding the role of PrP in health and disease.

O.16: Effect of cellular prion on neurogenesis after acute injury and chronic prion infection

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The cellular prion protein (PrP^C) has been associated with varied biological processes including cell signaling and neuroprotection, yet its physiological function(s) remain ambiguous. The goal of this study is to determine the role of PrP^C in adult neurogenesis using the murine olfactory system model. Olfactory sensory neurons (OSNs) within the olfactory sensory epithelium (OSE) undergo continual neurogenesis, integration, and turnover throughout adulthood, making it a useful model to study neuronal development. Here we determine the effect of PrP^C level on neurodevelopment in two injury models: acute injury and prion-induced neurodegeneration.

Acute nasotoxic injury was induced by methimazole injection and results in synchronized OSN regeneration. To investigate the role of PrP^C in OSN proliferation, dividing cells in the OSE were quantified using BrdU incorporation. Gene expression indicative of OSN differentiation was assessed by quantitative real-time PCR. Analysis revealed subtle effects of PrP^C on OSN differentiation and these altered gene expression patterns offer potential pathways to investigate PrP^C function in OSE neurogenesis. During prion infection there was an increase in nascent/immature OSN differentiation markers and a reduction in mature OSN gene expression. Additionally, the number of neural progenitor cells was initially increased, but over time fewer of those cells remained in the OSE. These findings suggest a loss of mature OSNs during prion infection

either by premature death or a deficiency in OSN maturation, and as a result, there is an increase in proliferation of neural progenitor cells to replenish the loss of mature OSNs.

O.17: Defining routes to neurodegeneration and the impact of the immune system

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In diseases such as Alzheimer's disease or prion diseases, aberrant folding of host encoded proteins acts as seeds for 'prion-like" propagation of normally folded protein to abnormal conformations. Misfolded proteins are regarded as causal factors of disease although their precise role in neurodegeneration remains unanswered. Activation of glial cells is an early pathological sign of disease, thus alterations in the status of these cells in the brain may impact on the disease process. Indeed viral infection has a controversial role in chronic neurodegeneration and has been implicated in both precipitating and driving the disease process.

Here we use prion models of neurodegeneration to demonstrate that prion disease can be both transmissible and non-transmissible. We examined the role of misfolded protein in the process of disease and in contrast to generally accepted selective spread of misfolded proteins, 'prion seeds' were widespread, distributed independently of neurodegeneration. Despite this, neurodegeneration and inflammatory responses were restricted to specific brain regions thus demonstrating that a misfolded protein seed is insufficient to initiate a neurodegenerative cascade.

We examined the role of glial cells and the impact of viral infection on the disease process and observe that a single co-infection event with a neurotropic viral agent has the ability to alter multiple aspects of disease, regional targeting, neuronal survival, inflammatory profile and biochemical properties of the misfolded prion protein. Such co-infection events can therefore have long term consequences for disease progression.

These data address the underlying mechanisms of neurodegeneration and identify new therapeutic targets for neurodegenerative diseases.

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P.01: Unusual case of sporadic Creutzfeldt-Jakob disease VV1 subtype

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Creutzfeldt-Jakob disease (CJD) is characterized by a variety of symptoms including rapidly progressive neurocognitive decline, ataxic gait, and myoclonus. Sporadic CJD (sCJD) has a median disease duration of 6 months. Based on the prion protein gene (PRNP) codon 129 polymorphism and prion protein type, seven different molecular subtypes of sCJD have been identified (MM1, MM2-C, MM2-T, MV1, MV2, VV1, VV2). The VV1 subtype is the rarest molecular subtype of sCJD (only about 1% of cases). To date, reported cases of sCJD VV1 have been characterized by young age at disease onset, an increased length of disease duration, and progressive neurocognitive degeneration. We describe a unique case of sCJD VV1 with older age at disease onset and short illness duration. VV1 is a challenging subtype to diagnose and this report adds further complexity and variation to its clinical characterization. Although PRNP codon 129 polymorphism and prion protein type exert a large influence on clinical and neuropathologic phenomenology in sCJD, other factors remain unknown and require further study.

P.02: A transfectant RK13 cell line permissive to caprine scrapie prion infection

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Classical scrapie is a transmissible spongiform encephalopathy that affects domestic goats and sheep. Animal bioassay can be performed to assess scrapie infectivity associated with caprine-origin tissues but incubation periods are long. No scrapie permissive cell line is currently available to study caprine scrapie. Therefore, the goals of this study were to generate a rabbit kidney epithelial cell line (RK13) stably expressing caprine wild type PRNP (cpRK13) and to assess its permissiveness to brain-derived caprine scrapie prion propagation. The cpRK13 and plasmid control RK13 cells (pcRK13) were incubated with scrapie brain inocula prepared from three wild type goats and two heterozygous goats (GS127 goat, or an IM142 goat). Significant accumulation of PrPSc was detected by ELISA in cpRK13 inoculated with wild type caprine scrapie inocula (3/3) but not incubation with the inocula derived from the heterozygous goats (0/2). However, PrPSc accumulation levels were improved in cpRK13 inoculated with ovinized transgenic mice (Tg338) passaged

caprine scrapie samples when compared to the original heterozygous caprine scrapie inocula. Accumulation of PrPSc in wild type caprine scrapie inoculated cpRK13 cells could also be detected by scrapie immunohistochemistry. Western blot analysis revealed typical di-, mono- and un-glycosylated proteinase K-resistant PrPSc isoforms in wild type caprine scrapie inoculated cpRK13 lysate. Importantly, PrPSc accumulation was not detected in similarly inoculated pcRK13 cells, whether by ELISA, western blot, or scrapie immunohistochemistry. Taken together, these findings suggest that cpRK13 may be a useful model to assess caprine classical scrapie in brain tissues.

P.03: Visual art therapy in sporadic Creutzfeldt-Jakob disease: A case study

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We describe the diagnostic and treatment utility of visual art therapy in a case of sporadic Creutzfeldt-Jakob disease. Visual art therapy was collected and compared longitudinally with clinical and neuroimaging data over a course of 5 months in an autopsy confirmed case of sporadic Creutzfeldt-Jakob disease of the MM2-cortical subtype. The visual art treatment sessions and ensuing content was useful in ascertaining neuropsychiatric symptoms during the course of her illness. Art therapy also offered a unique emotional and cognitive outlet for patient and family as her illness progressed. Patients and families affected by sporadic Creutzfeldt-Jakob disease may benefit from art therapy despite the rapid and progressive nature of the illness. Art therapy can also be useful for the assessment of patients with sporadic Creutzfeldt-Jakob disease by healthcare professionals.

P.04: Conduct of diagnosis in the case of occurrence of bovine spongiform encephalopathy in Romania

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Bovine spongiform encephalopathy (BSE) is a fatal infectious disease neurodegenerative, caused by prions that affects mainly cattle, characterized by spongiform and vacuolar changes in the central nervous system (CNS). Although in the last period of time, the appearance of the classic cases of BSE had fallen, is the appearance of atypical BSE forms, in many countries of Europe, USA, Japan and Canada. The paper presents the conduct of diagnosis for the first cases of BSE in Romania, reported on the basis of laboratory investigations, the rapid test positive to bovine animals slaughtered normally diagnosed after confirmatory tests carried out by the National Reference Laboratory for Transmissible Spongiform Encephalopathies (NRL-TSEs) within the Institute for Diagnosis and Animal Health (IDAH). After carrying out tests for the differentiation of strains of BSE samples sent to the European Union Reference Laboratory for the Transmissible Spongiform Encephalopathies (EU-RL-TSE) from the Animal and Plant Health Agency (APHA) Weybridge, United Kingdom, were diagnosed 2 cases of atypical BSE, type L.

P.05: In search for a mammalian disaggregase function involved in prion propagation

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Prion diseases are a family of transmissible fatal neurodegenerative disorders that affect both human and animals. They are characterized by conformational conversion of the normal cellular prion protein (PrP^{c}) into the disease associated isoform PrP^{Sc}. Accumulation of PrP^{Sc} aggregates leads to brain damage. The fragmentation of the aggregates into smaller infectious seeds is considered a requirement for prion propagation, a process which involves disaggregase function. The responsible mammalian disaggregase was not yet identified. The role of autophagy, a basic cellular degradation machinery, has been addressed in prion diseases by us and others. Our data in mouse embryonic fibroblasts (MEFs) show that homozygous knockout of the autophagy gene Atg5 blocking autophagic flux results in an inability to effectively propagate PrP^{Sc}. On the other hand, wild-type MEFs showed increased levels of the autophagy marker LC3-II when they started propagating PrP^{Sc}. These data demonstrate that a basal level of autophagy is required for initiating a primary prion infection. Recently, it became a subject of intensive research how the endosomal sorting complex required for transport (ESCRT) system is involved in completion of autophagy. Our confocal microscopy data showed that persistently infected neuronal cells (ScN2a) transfected with Rab 7, 9 or 11 (ESCRT system members) harbor more PrP^{Sc} aggregates compared to those transfected with wild-type constructs. Interestingly, a transient knockdown of autophagy using siRNA against Atg5 increased PrP^{Sc}aggregates compared to control treated ScN2a cells. Taken together, our data demonstrate a possible role for ESCRT and autophagic machineries as a disaggregase in PrP^{Sc} formation.

P.06: Assessing mother to offspring transmission of chronic wasting disease using a transgenic mouse model

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Chronic wasting disease (CWD) is the transmissible spongiform encephalopathy (TSE), of free-ranging and captive cervids (deer, elk and moose). The presence of infectious prions in the tissues, bodily fluids and environments of clinical and preclinical CWD-infected animals is thought to account for its high transmission efficiency. Recently it has been recognized that mother to offspring transmission may contribute to the facile transmission of some TSEs. Although the mechanism behind maternal transmission is not yet known, the extended asymptomatic TSE carrier phase (lasting years to decades) suggests that it may have implications in the spread of prions.

Placental trafficking and/or secretion in milk are 2 means by which maternal prion transmission may occur. In these studies we explore these avenues during early and late infection using a transgenic mouse model expressing cervid prion protein. Naïve and CWD-infected dams were bred at both timepoints, and were allowed to bear and raise their offspring. Milk was collected from the dams for prion analysis, and the offspring were observed for TSE disease progression. Terminal tissues harvested from both dams and offspring were analyzed for prions.

We have demonstrated that (1) CWD-infected TgCerPRP females successfully breed and bear offspring, and (2) the presence of PrP^{CWD} in reproductive and mammary tissue from CWD-infected dams. We are currently analyzing terminal tissue harvested from offspring born to CWD-infected dams for the detection of PrP^{CWD} and amplification competent prions. These studies will provide insight into the potential mechanisms and biological significance associated with mother to offspring transmission of TSEs.

P.07: The environmental neurotoxicant manganese promotes prion-like cell-to-cell transmission of α-synuclein via exosomes in cell culture and animal models of Parkinson's disease

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The aggregation of α-synuclein (αSyn) is considered a key pathophysiological feature of Parkinson's disease (PD). Recent studies suggest that a prion-like cell-to-cell transfer of misfolded αSyn contributes to the spreading of αSyn pathology. However, biological mechanisms underlying the propagation of the disease with respect to environmental neurotoxic chemical exposures not well understood. Considering role of the divalent metal manganese in PD-like neurological disorders, we characterized its effect on αSyn misfolding and protein aggregation. First, we established an MN9D dopaminergic cell line stably expressing wild-type human αSyn and treated it with non-toxic doses of manganese at multiple time points. Analysis of condition medium (CM) through Western blot showed that cells secreted αSyn into extracellular media following manganese exposure. Further characterization of CM through electron microscopy readily detected nano-sized vesicles with the characteristic hallmarks of exosomes. Western blot and ELISA studies revealed that the exosomes do in fact contain αSyn. Furthermore, Nanosight particle analysis showed that manganese exposure enhances the release of αSyn-containing exosomes. In functional studies, we demonstrated that exosomes released during manganese treatment can induce neuroinflammatory responses in primary microglial cultures and neurodegeneration in differentiated human dopaminergic cells (LUHMES) through the activation of caspase-3 signaling. We also showed for the first time that stereotaxic delivery of αSyn-containing exosomes isolated from manganese-treated αSyn-expressing cells into the striatum can initiate PD-like motor deficits in mice. Collectively, these results demonstrate that manganese exposure promotes αSyn secretion via exosomal vesicles, which subsequently evoke pro-inflammatory and neurodegenerative responses in both cell culture and animal models.

P.08: Two Korean families with Gerstmann-Sträussler-Scheinker disease

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Gerstmann-Sträussler-Scheinker disease (GSS) is a type of human transmissible spongiform encephalopathy that is determined genetically. We report 2 Korean families with GSS.

Case 1 A 46-year-old woman was admitted due to a slowly progressive ataxic gait and speech disturbance that had started 3 y earlier. On examination, she revealed abnormal Tandem gait and slight dysmetria. Cognitive decline developed one year prior to admission. She had been bedridden for the 2 months prior to her admission. In the family history, 2 sisters out of 7 siblings developed similar symptoms in their fourth and fifth decade, respectively, and both expired approximately 5 y after the onset of symptoms. **Case 2** A 31-year-old woman presented with unsteady gait, dysarthria and dizzy sense which gradually progressed. Six months later, she could not walk independently. At the same time, severe memory impairment and incoherent speech were noticed. No later than one month, she was bedridden and could respond only with simple words. Her mother showed similar features and 5 family members over 4 generation were suspected to have similar conditions. Both cases

revealed high signal intensities over entire cortex in diffusion weighted imaging. PRNP analysis revealed a mutation in codon 102 proline to leucine.

Conclusions. GSS should be considered for differential diagnosis when hereditary cerebellar ataxia and progressive cognitive decline develops. Unlike CJD, GSS is characterized by long periods of illness, and dementia develops late in the course of the illness. The utilization of diffusion-weighted MRI is suggested for the early diagnosis of GSS.

P.09: LPS-induced systemic inflammation in goats naturally devoid of prion protein

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Although a large body of knowledge support the obligate function of the prion protein (PrP) in human and animal prion disorders, evidence of the normal function of PrP remains elusive. However, expression across tissues in mammalian species suggests that it may be involved in a variety of physiological functions. Recently, a nonsense mutation blocking the expression of PrP in healthy Norwegian Dairy Goats was discovered as the first report of naturally-occurring PrP-free animals.

To investigate the role of PrP in the inflammatory response, we plan a case-control study of lipopolysaccharide (LPS)-induced inflammation in homozygote ($PRNP^{Ter/Ter}$) goats carrying this mutation, compared to normal goats ($PRNP^{+/+}$) expressing PrP. In a preliminary study, 2 $PRNP^{+/+}$ goats and one $PRNP^{Ter/Ter}$ goat were intravenously challenged with LPS (*Escherichia coli O26:B6*) twice. Two *PRNP*+/+ goats received corresponding volumes of saline. Clinical and behavioral parameters were monitored for 30 hours and blood samples were collected during the experiment. After euthanization, tissue samples for histopathology, freezing and expression analysis were collected.

Characteristic clinical signs of acute sepsis accompanied by marked decrease of peripheral leukocytes were observed after LPS administration. Fever was most prominent in the $PRNP^{Ter/Ter}$ goat. In neurons of hippocampal dentate gyrus, single cell necrosis were evident in animals receiving LPS. Lung edema and subpleural bleedings were observed in the $PRNP^{Ter/Ter}$ goat, microscopically accompanied by neutrophil leukostasis, increased amount of alveolar macrophages and alveolar bleedings.

Our initial findings support that this LPS model is suitable for further work elucidating PrP functions.

P.10: Recapitulation of prion disease pathology and abnormal PrP deposition patterns in organotypic cerebellar slices

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Organotypic cerebellar slices exhibit largely preserved tissue architecture and represent a suitable model for characterizing and manipulating prion replication in a complex cell environment. However, the cellular distribution of disease specific PrP^d in organotypic slices has not been assessed. Here we report the simultaneous detection of disease-specific prion protein PrP^d and CNS markers in cerebellar slices of C57BL/6 pups infected with mouse-adapted prion strain 22L. Despite substantial abnormal PrP accumulation, no significant decrease in viability or mitochondrial respiration was observed over the course of 11.5 weeks. Still, epifluorescence and confocal microscopy of intact, unsectioned slices revealed key

hallmarks of TSE pathology, including focal loss of purkinje cell dendrites, spongiform degeneration in the molecular layer, and reactive astro- and microgliosis. Unmasking of PrP epitopes allowed for the specific detection of pathologic PrP by immunocytochemistry. PrP^d distribution profiles in organotypic slices closely resembled those *in vivo*, demonstrating granular spot like deposition predominately in the molecular and purkinje cell layer. Double immunostaining of PrP^d and CNS cellular markers identified PrP^d in the neuropil and associated with astrocytes and microglia, but clear absence of PrP^d in purkinje cells. The established protocol for the simultaneous immunocytochemical detection of PrP^d and cellular markers in intact > 200 μm slices enables detailed analysis on prion replication in a complex *ex vivo* system.

P.11: Clinical features in Gerstmann-Sträussler-Scheinker syndrome with P105L mutation

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Introduction. Gerstmann-Sträussler-Scheinker syndrome (GSS) with P105L mutation in the prion protein gene (*PRNP*; GSS105) has been reported only from Japan. The Clinical features have not been clarified because of its very low predisposition.

Patient and Methods. We statistically analyzed clinical symptoms and laboratory findings of GSS105 in the data collected by Japanese Prion Disease Surveillance between April 1999 and September 2013, and compared them with those of GSS with P102L mutation in *PRNP* (GSS102).

Results. All 14 GSS105 cases, 8 males and 6 females, had family history. Mean age at onset was 48.1, which was younger than that of GSS102 (Kruskal-Wallis: $p = 0.09$). Most predominant initial symptom was extrapyramidal (50%), followed by slowly progressive dementia (14%) and body pain (14%), and cerebellar symptom was quite rare (7%). Ninety %, and 75 % of the patients with GSS105 showed slowly progressive dementia and extrapyramidal symptoms, respectively. Periodic sharp wave complexs (PSWCs) were not detected in electroencephalograms (EEGs) of all patients, even in patients showing myoclonus (36%). High intensity signal lesions in diffusion-weighted imaging of MRI were detected in 14% of the patients. All the patients had 129 MV heterozygosity, with the 129 V codon on the same allele as the 105 L.

Conclusion. Clinical feature of GSS105 was characterized by slowly progressive cognitive impairment and extrapyramidal signs without PSWCs in EEGs and with low frequency of high signal intensity in MRI.

P.12: MALDI-TOF mass spectrometry for analysis of polymorphisms in the genome sheep for the detection of susceptibility to scrapie

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The objective of this study was to compare the field performances between 2 different methods, MALDI-TOF mass spectrometry and RT PCR for analysis of polymorphisms in the genome sheep 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 is 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 MALDI-TOF mass spectrometry, designated DNA MassARRAYTM, has been developed. DNA MassARRAYTM uses samples in chip-based, highdensity arrays. This system accurately calls SNPs in individual DNA samples, or alternatively determines SNP allele frequencies in DNA pools. Assay design for MassARRAYTM is simple, flexible and has been automated to allow designing vast numbers of assays, all of which can be run using a universal set of reaction conditions.

P.13: Detection of sCJD prions in human saliva by RT-QuIC

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Sporadic Creutzfeldt-Jakob Disease (sCJD) is the most common form of human prion disease. A recent study showed that prion seeding activity is RT-QuIC-detectable in the olfactory neuroepithelium of sCJD patients. Relatively rapid turnover of the olfactory neuroepithelium and nasal mucus clearance systems might lead to the transportation of prion seeds into the oral cavity and shedding through saliva. Pooled human saliva was spiked with sCJD prions and subjected to different treatments to investigate the suitability of such a sample as a new and non-invasive diagnostic specimen. Our findings highlighted the presence of yet unidentified factor(s) that could lead to spontaneous conversion in the RT-QuIC assay. We will show our ongoing results on the attempt to identify the factor (s) and eliminate it/them.

P.14: Plant-derived vaccine against chronic wasting disease using multimeric deer PrP^C and its derivatives as immunogens

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Chronic wasting disease (CWD) is a prion disease that affects free-ranging and farmed cervids. It is the most contagious prion disease, transmissible with direct animal to animal contact or indirect exposure to prions in the environment. The prevalence of CWD has increased rapidly in North America and high infection rates can be seen in captive and free-ranging cervids. Thus, it is apparent that there is an urgent need to control and prevent this disease from spreading.

One potential means of controlling CWD is through active vaccination using cellular prion protein (PrP^C) as the immunogen. Moreover, one technology receiving attention is the use of plant-based expression systems to produce recombinant proteins as vaccines. This platform provides an economical and viable alternative to other vaccine production systems such as microbial and cell culture systems. Plants act as natural bioreactors with infrastructure for cultivation and harvesting readily available. Vaccines can stably accumulate in seed plants such as *Arabidopsis thaliana* and *Brassica napus* (Canola) and the seeds can be transported and stored at ambient temperature without refrigeration.

Our strategy is to produce a plant-derived vaccine using cervid multimeric PrP^C. Our preliminary results show that plant-derived deer PrP^C multimers can stably accumulate in seeds of *A. thaliana* plants. To our knowledge this is the first report of expression of recombinant PrP^C in a plant system. Several transgenic lines are currently being scaled-up for biochemical analysis and immunization experiments. Our overall goal is to develop an effective vaccine against CWD and prion-related diseases.

P.15: Characterization of differential proteome expression of MM1 and VV2 subtypes of sporadic Creutzfeldt-Jakob disease (sCJD) in Cerebellum

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Keywords. neuro-degeneration, prion diseases, sporadic Creutzfeldt-Jakob disease,

Sporadic Creutzfeldt-Jakob disease (sCJD) is an example of human prion diseases with idiopathic in its origin[1]. Codon 129 genotype of PrP plays an important role in determining the susceptibility of different parts of the brain to neuro-pathological events in sCJD[2]. VV2 subtype shows an elevated expression of total and un-glycosylated PrP protein, distinct PrP plaque-like deposition and IL-6 and TNF-α at mRNA level as compared to MM1 subtype only in Cerebellum part of the brain. An increased expression of GFAP along with variable expression of microglia in both subtypes MM1 and VV2 of sCJD and more microvacuoles based spongiform degeneration in MM1 subtype is seen in the cerebellum of sCJD[3]. In this study, we aimed to characterize differential proteome expression of MM1 and VV2 subtypes of sCJD in Cerebellum part of the brain by using 2-Dimensional gel electrophoresis. We had found total of 38 regulated protein spots between MM1 and control

samples, 43 regulated protein spots between VV2 and control samples and only 9 regulated protein spots between MM1 and VV2 samples. Further identification of these differentially regulated protein spots by Q-TOF MS/MS and then validation of identified regulated proteins by western blot was done. Altogether, these results indicate the codon 129 genotype of PrP based proteomic alterations and role of PrP^C in neurodegeneration in prion diseases in general and in sporadic Creutzfeldt-Jakob disease in particular which may help to discover some early novel diagnostic markers and therapeutic strategies as well.

P.16: Glycosaminoglycan modulation affects cellular prion replication downstream of PrP^{Sc} internalization

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Prions are unconventional infectious agents composed primarily of misfolded aggregated host prion protein PrP, termed PrP^{Sc}. Conversion of cellular prion protein into PrP^{Sc} occurs on the cell surface or along the endocytic pathway. The precise mechanisms and cellular requirements for PrP^{Sc} uptake, the initial PrP^{Sc} formation and the persistent PrP^{Sc} propagation still remain unknown. Glycosaminoglycans (GAGs), highly-sulfated unbranched polysaccharides present on the cell surface and within endocytic vesicles, have been implicated as first attachment sites for prions and cofactors for replication. GAG mimetics and obstruction of GAG sulfation affect prion replication, but so far, comparative analysis of the role of GAGs during the individual stages of infection by 22L prion strain has not been performed. We examined the effect of the GAG mimetic, DS-500, and the sulfation inhibitor, $NaClO_3$, on prion infection by scrapie strain 22L in L929 cells and organotypic cerebellar slices. Here we show that both compounds change the cellular distribution and levels of sulfated GAGs but have divergent effects on cell surface and total PrP^C levels in L929 cells. Chemical manipulation of GAGs did not prevent PrP^{Sc} uptake, arguing against their role as essential attachment sites. Importantly, GAG undersulfation and DS-500 effectively antagonized *de novo* and chronic 22L prion infection in L929 cells and organotypic cerebellar slices. We conclude that DS-500 and $NaClO_3$ affect events downstream of the initial PrP^{Sc} attachment and internalization.

P.17: High throughput detection of PrP^{Sc} from prion-infected cells without PK treatment: Cell-based ELISA for novel screening method for anti-prion compounds

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Screening of chemical libraries is one of the possible ways for identification of therapeutic compounds for prion diseases. Prion-infected cells are often used for analyzing the effect of compounds on PrP^{Sc} formation. Mostly, PrP^{Sc} is detected by anti-PrP antibodies after a removal of PrP^C by proteinase K (PK) treatment. However, PK-sensitive part of PrP^{Sc} (PrP^{Sc}-sen) that possesses higher infectivity and conversion activity than the PK-resistant PrP^{Sc} (PrP^{Sc}-res) is expected to be also digested by PK treatment. To overcome this problem, in

this study, we attempted to establish a novel cell-based ELISA for screening of anti-prion compounds, in which PrP^{Sc} can be directly detected from prion-infected cells without PK treatment. The mAb 132 that recognizes epitope consisting of mouse PrP aa 119–127 enabled us to detect PrP^{Sc} directly from prion 22L strain-infected Neuro2a cells (N2a) pretreated with GdnSCN without PK treatment. PrP^{Sc} also could be detected from Chandler strain-infected N2a cells and 22L strain-infected GT1–7 cells. Analytical dynamic range for PrP^{Sc} detection was about 1Log. The coefficient of variation and signal to background ratio were 711%– and 2.5–3.3, respectively, demonstrating the reproducibility and stability of this assay. The addition of WST assay for evaluating cytotoxicity of compounds did not influence the following PrP^{Sc} detection, therefore, all the procedures including culture, cytotoxicity assay and PrP^{Sc} detection can be completed in the same plate. The simplicity and no requirement for PK treatment of the novel cell-based ELISA appear to be remarkable advantages for high screening of anti-prion compounds.

P.18: Attempts to amplify Nor98 *in vitro* by bank vole PMCA

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

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

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

P.19: Characterization of chronic wasting disease isolates from free-ranging deer (*Odocoileus sp*) in Alberta and Saskatchewan, Canada

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Chronic wasting disease (CWD) is an emerging prion disease of free ranging and captive species of *Cervidae*. In North America, CWD is enzootic in some wild cervid populations and can circulate among different deer species. The contagious nature of CWD prions and the variation of cervid *PRNP* alleles, which influence host susceptibility, can result in the

emergence and adaptation of different CWD strains. These strains may impact transmission host range, disease diagnosis, spread dynamics and efficacy of potential vaccines. We are characterizing different CWD agents by biochemical analysis of the PrP^{CWD} conformers, propagation *in vitro* cell assays[1] and by comparing transmission properties and neuropathology in *Tg33* (Q95G96) and *Tg60* (Q95S96) mice.[2] Although *Tg60* mice expressing S96-PrP^{C} have been shown resistant to CWD infectivity from various cervid species,[2,3] these transgenic mice are susceptible to $H95^{+}$ CWD, a CWD strain derived from experimental infection of deer expressing H95G96-PrP^{C}. The diversity of strains present in free-ranging mule deer (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginianus*) from Alberta and Saskatchewan is being determined and will allow us to delineate the properties of CWD agents circulating in CWD enzootic cervid populations of Canada.

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P.20: Efficient *in vivo* propagation of PrP amyloids in transgenic mice expressing anchorless prion proteins

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PrP^{Sc} displays structural diversity, ranging from small oligomers to large aggregates. It was shown that non-amyloidogenic PrP prion strains could be converted into amyloidogenic prions by inoculation into transgenic mice expressing anchorless PrP [Tg(PrP*ΔGPI)]. Following inoculation of Tg(PrP*ΔGPI) mice with RML prions, we observed a substantial shortening of the incubation periods upon serial passaging. This was accompanied by the accumulation of fold5- more PK-resistant, anchorless PrP in second and third passages. Following these passages, anchorless PrP prions were passaged into mice expressing wt PrP^{C}. Although still transmissible, we observed that the prion titer dropped $\sim 10^4$ ID_{50} units, suggesting that only a small fraction of anchorless PrP was converted into PrP^{Sc} structure while most of the anchorless PrP assembled into amyloid fibrils that were distinct from PrP^{Sc}. Furthermore, inoculation of amyloidogenic recombinant (rec) PrP (residues 23–231 and 89–230) into Tg(PrP*ΔGPI) mice also resulted in signs of neurologic dysfunction within 200–300 d postinoculation. Serial transmission of synthetic, anchorless PrP prions in Tg(PrP*ΔGPI) mice further shortened the incubation time. Compared to GPI-anchored, RML prions upon primary passage, incubation times for anchorless prions in Tg(PrP*ΔGPI) mice were 200–300 d shorter. We suggest that this difference in the susceptibility of Tg

(PrP*ΔGPI) mice to prions derived from recPrP amyloids and natural sources such as sheep scrapie is due to structural differences that were found in fiber diffraction studies. In summary, our studies demonstrate the profound differences in biological properties of prions with or without a GPI anchor.

P.21: Structural analysis of the anti-scrapie activity of DB772 in a persistently-infected ovine microglia culture system

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Despite efforts to identify anti-prion compounds, an effective therapeutic does not exist. The *in vitro* inhibitory activity of several compounds has failed to translate *in vivo*, which may be attributed to use of systems adapted to lab animals. The purpose of this study was to determine the structural basis for the anti-prion activity of a novel, monocationic phenyl-furan-benzimidazole (DB772) by screening an existing library of related structure-substituted compounds in a scrapie microglial cell culture system. Eighty-9 compounds, including DB772, were tested at 1μM in human telomerase-immortalized ovine microglia. Anti-prion activity was determined by the Standard Scrapie Cell Assay. The effects of compounds on cell growth and PrP^{C} content were respectively identified by WST-1 assay and ELISA. The effect of DB772 on PrP^{Sc}-seeded misfolding was determined by PMCA.

Thirteen compounds were considered too toxic for further study. Seven compounds were nontoxic and reduced PrP^{Sc} accumulation by $\geq$ ½-log. Anti-prion activity appeared dependent on a central 5-membered heterocyclic ring. Activity in such molecules was variably affected by the combinations of benzimidazole and imidazole side groups, and terminal amidines. The greatest anti-prion activity was associated with symmetrical bis-benzimidazole structures containing a 5-membered heterocyclic aromatic ring. Active compounds did not appear to affect total PrP^{C}. Preliminary results using DB772 show concentration-dependent inhibition of PrP^{Sc}-seeded misfolding. In conclusion, insights into the structural determinants of the anti-scrapie activity of DB772-related compounds were identified. Active compounds did not appear to effect total cellular content of PrP^{C}, but may act by reducing conversion of PrP^{C} to PrP^{Sc}.

P.22: Conformational dynamics of the cellular prion protein

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Prions are infectious agents responsible for transmissible spongiform encephalopathies, a fatal neurodegenerative disease in mammals, including humans. Prions propagate biological information by conversion of the nonpathological version of the prion protein, PrP^{c}, to the infectious conformation, PrP^{Sc}. As a first step to decipher the prion conversion mechanism, we aim at understanding the equilibrium conformational dynamics of the cellular isoform.

We will report on structural bioinformatics and multiscale molecular modeling studies to investigate the interplay between PrP^c topology and fragment amylogenicity; and the modulation on the observed behavior by the local milieu, i.e. bulk vs. cell surface.

P.23: An improved test for the detection of Creutzfeldt-Jakob Disease from human CSF using new RT-QuIC conditions

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Neurodegenerative protein misfolding diseases are difficult to diagnose early and accurately. This is particularly worrisome with human prion diseases, such as Creutzfeldt-Jakob disease (CJD), because prions are transmissible, deadly, and unusually resistant to decontamination. Real-time quaking-induced conversion (RT-QuIC) assays allow highly sensitive and specific testing for CJD using human cerebrospinal fluid (CSF) or nasal brushings and are being widely implemented as important diagnostic tools. However, such laboratory analyses have required 2.5 to 5 d to complete. Furthermore, CSF testing using previously evaluated RT-QuIC conditions still yields false negative results in 11 to 23% of CJD cases. We have now developed an improved RT-QuIC assay which can identify positive CSF samples within 4 to 14 h with better analytical and diagnostic sensitivity. Analysis of CSF samples from 11 CJD patients demonstrated that while 7 were RT-QuIC positive using previous conditions, an additional 3 samples were positive using the new assay. In these and subsequent analyses, a total of 46 of 48 CSF samples from sporadic CJD patients gave positive RT-QuIC responses, while all 39 non-CJD patients were negative, giving 95.8% diagnostic sensitivity and 100% specificity. This diagnostic sensitivity was significantly better than that obtained using the previous conditions. We continue to expand the testing of CJD-positive and -negative CSF samples to further establish the diagnostic utility of this new assay for various human prion diseases. So far, our improved RT-QuIC assay appears to allow for much faster, more accurate and practical antemortem testing for CJD using CSF samples.

P.24: A comparative study of dura mater graft-associated Creutzfeldt-Jakob disease between Japan and other countries

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Objective. More than 60% of patients worldwide diagnosed with Creutzfeldt-Jakob disease

(CJD) associated with dura mater graft (dCJD) have been identified in Japan. The remarkable frequency of dura mater graft use in Japan might contribute to the elevated incidence of dCJD, but the possible reasons for the disproportionate use of this procedure in Japan remain unclear. We investigated the differences between dCJD patients in Japan and those elsewhere to help uncover an explanation for the unusually more frequent use of cadaveric dura mater and high incidence of dCJD in Japan.

Methods. We obtained data of dCJD patients in Japan from the nationwide surveillance of CJD in Japan and of those in other countries from extant literature. We compared demographic, clinical, and pathological features of dCJD patients between Japan and elsewhere.

Results. Data from 142 dCJD patients in Japan and 53 in other countries were obtained. The medical conditions precipitating dura mater graft were significantly different between Japan and other countries ($P < 0.001$); in Japan, there were more cases of cerebrovascular disease and hemifacial spasm or trigeminal neuralgia. Patients with dCJD in Japan received dura mater graft more often for non-life-threatening conditions, such as meningioma, hemifacial spasm and trigeminal neuralgia, than those in other countries.

Conclusion. Differences in the medical conditions precipitating dura mater graft may contribute to the frequent use of cadaveric dura mater and the higher incidence of dCJD in Japan.

P.25: Subcutaneously administered LPS-converted recombinant mouse prion protein alone or in combination with LPS modulates the content of prion-related proteins in the brain of FVB/N mice

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We tested whether subcutaneous (sc) injection of LPS-converted mouse moPrP, which is resistant to proteinase K digestion ($moPrP^{res}$), was able to affect concentrations of prion-related proteins including PRNP, SPRN, and PRND in the brains of wild type FVB/N female mice. We also explored the effect of using $moPrP^{res}$ alone or in combination with *E. coli* LPS. Five groups of mice (n = 15) were treated sc either with saline (CONtrol) or lipopolysaccharide (LPS), or with PrP^{res}, PrP^{res} plus LPS, RML, and RML plus LPS. Saline and LPS were injected chronically for 6 weeks by ALZET minipumps; whereas PrP^{res} and RML were administered as a one time injection at the beginning of the experiment. Mice were allowed to develop neurodegenerative disease and euthanized at the terminal stage. Brain homogenates were analyzed with ELISA kits to measure the concentrations of PRNP, SPRN, and PRND. Results showed that all treatments increased concentrations of PRNP with $moPrP^{res}$ increasing it more than fold3- and RML almost fold5-. Combinations of $moPrP^{res}$ with LPS and RML with LPS, or LPS alone, elevated concentrations of PRNP versus saline. In addition, $moPrP^{res}$ increased SPRN almost fold6- and treatment with $moPrP^{res}$ and LPS more than fold15- compared to CON. Treatment with RML increased SPRN 2.fold8-, whereas combination of RML with LPS enhanced the amount of SPRN 1.fold4- vs saline. $moPrP^{res}$ increased PRND concentration fold2- and RML fold3- vs CON. Additionally, $moPrP^{res}$ and RML with LPS and LPS alone increased concentrations of PRND in the brain

homogenates of mice compared to CON animals.

P.26: Allele-specific RNA interference (RNAi) mitigates pathology in the Tg (PrP-A116V) mouse model of Gerstmann-Sträussler-Scheinker disease (GSS)

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Allele-specific RNAi is an attractive therapeutic approach for genetic prion diseases, as it targets the pathogenic allele, thereby limiting potential problems of long-term wild type (wt) PrP suppression. Our Tg(PrP-A116V) mice model human GSS(A117V) and develop the cardinal features of progressive gait ataxia and PrP amyloid plaque accumulation, primarily within the cerebellum. We, therefore, designed siRNAs differing in length (19 or 21 nucleotides) and position of the target mutation sequence (5' end, mid-position, or 3' end) and tested the selectivity and efficacy of each to inhibit expression of PrP-116V in transfected COS-7 and N2a cells, for eventual application in Tg(PrP-A116V) mice. A 19 nt siRNA with the target sequence at the 3' end displayed the best profile. This was prepared as a short hairpin (sh) RNA and incorporated into a lentiviral vector that was injected into the cerebellum of 45 day-old Tg(PrP-A116V) mice. Compared with empty vector control (C), lentivirus carrying active (A) shRNA reduced cerebellar PrP-116V mRNA by $62.3 \pm 6.8\%$ ($p < 0.01$) and PrP plaque burden by 72.2 ± 2.2 % ($P < 0.01$), although disease onset [A = 130 .0 ± 11.3 vs. C = 123 .2 ± 16.1 d] and death [A = 164 .4 ± 16.6 d vs. C = 159 .5 ± 21 .3 d], were only slightly, but not significantly, delayed. Equivalent injections in wt mice did not affect wt-PrP mRNA. These results are encouraging, as the reduction of cerebellar histopathology predicts a likely clinical benefit with expanded CNS expression of the shRNA. They also highlight the importance of non-cerebellar brain regions in the development of the clinical phenotype of GSS.

P.27: Intracranial injection of LPS-converted mouse recombinant PrP alone or with LPS causes brain neurodegeneration in FVB/N mice

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Recently we reported that lipopolysaccharide (LPS) from *Escherichia coli* 0111:B4 is able to convert the mouse prion protein into a b-rich isoform resistant to proteinase K (moPrP^{res}). We also tested whether intracranial (ic) administration of moPrP^{res} (29–232) is able to cause prion-like pathology *in vivo*. Sixty five FVB/N female mice were treated ic with 30 μL of: 1) 1% NBH (CON) 2) LPS (0.5 mg/mL), 3) moPrP^{res} (0.5 mg/mL), 4) moPrP^{res}+LPS (0.5 mg/mL each), and 5) 1% RML (10^7 ID units). Thirteen animals died during ic, including 7 from LPS and 4 from RML treatment. Animals that survived ic injection were left to develop clinical signs of disease until terminal sickness or terminated at 589 post inoculation without clinical signs. Hematoxilin and eosin (H&E) and PrP^{Sc}-stainings were conducted to determine presence of vacuolation and PrP^{Sc} accumulation in the brains, respectively. All treatment groups, except for saline, showed brain vacuolation in the cerebral cortex (Cc), thalamus (Th), midbrain (Mb), and cerebellum (Cr) and mild PrP^{Sc} accumulation only in the RML group. Larger number of vacuoles were found in Th and Cr regions of moPrP^{res}, PrP^{res}+LPS, and RML+LPS treated mice vs the control (CON) group. Greater neurodegeneration of Cc white matter was observed in moPrP^{res} and PrP^{res}+LPS vs RML group. The PrP^{Sc}-staining showed PrP^{Sc} accumulation only in the RML group. In conclusion moPrP^{res}, LPS, and PrP^{res}+LPS treated mice developed

brain neurodegeneration at terminal sickness. Treatment with LPS and PrP^{res}+LPS seems to cause larger vacuoles in the brain compared to the RML-only group.

P.28: Proteins are recruited into polyglutamine aggregates via their intrinsically-disordered domains

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Intracellular protein aggregation is the hallmark of several neurodegenerative diseases. Aggregates formed by polyglutamine (polyQ)-expanded proteins, such as Huntingtin, assume amyloid-like structures that are resistant to denaturation. We combined mass spectrometry and a stringent purification procedure to identify the protein species that are trapped within aggregates formed by Huntingtin N-terminal fragments with pathogenic polyQ tracts (> 40 glutamines) in both yeast and mammalian (PC12) cells. We found that protein quality-control and RNA-binding proteins were greatly enriched in polyQ aggregates, and despite their evolutionary divergence, there was significant conservation between trapped proteins identified from yeast and PC12 cells. Notably, in the mammalian cells, a number of neurodegenerative disease-linked proteins were consistently found trapped in the polyQ aggregates. Many of these proteins are found in neuronal inclusions in their respective diseases, suggesting that polyQ aggregates can recruit proteins that are prone to aggregation in different pathological contexts. We also analyzed the primary and secondary structure of our aggregate-associated proteins and discovered a significant enrichment of proteins with very long intrinsically-disordered (ID) domains. When we truncated the ID domains of selected proteins, the proteins no longer co-aggregated with polyQ. The high frequency of ID domains in RNA-binding proteins may explain why these proteins are disproportionately found in pathological inclusions in many neurodegenerative diseases.

P.29: Bile acids reduce prion conversion and neuronal death in models of prion disease

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No treatment is available for prion diseases. The bile acids tauroursodeoxycholic acid (TUDCA) and ursodeoxycholic acid (UDCA) have been recently shown to be neuroprotective in models of other protein misfolding diseases like Parkinson's, Huntington's and Alzheimer's diseases. We studied the therapeutic efficacy of these compounds in prion disease models. We demonstrated that TUDCA and UDCA substantially reduced PrP conversion in cell-free aggregation assays as well as in chronically and acutely infected cell cultures. This effect was mediated through a reduction of the seeding ability of PrPSc, rather than an effect on PrPC. We also demonstrated the ability of TUDCA and UDCA to prevent neuronal loss in prion infected cerebellar slice cultures. In addition, we found that the levels of the protein PSD-95 were modulated by the treatment and this modulation was independent from the inhibitory effect of bile acids on PrPSc accumulation. Lastly, we demonstrated that low doses of UDCA reduced the levels of GFAP-driven luciferase induced by prion infection in GFAP-luc mice and prolonged the lifespan of infected C57Bl6 mice. Interestingly, these

effects were limited to the males, implying a gender-specific difference in drug metabolism. Thus, as demonstrated for other neurodegenerative diseases, we propose that TUDCA and UDCA may have a therapeutic role in prion diseases, with effects on both prion conversion and neuroprotection. Our findings, together with the fact that these natural compounds are orally bioavailable, permeable to the blood-brain barrier and FDA-approved for use in humans, make these compounds promising alternatives for the treatment of prion diseases.

P.30: Identification of Q,N-rich transcription factors that increase the synthetic lethality of prion [*PSI*$^+$] with *sup45* mutations in *Saccharomyces cerevisiae*

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Previously, we proposed a synthetic lethality test for genes that may influence the properties of translation termination factors Sup35 and Sup45. It is based on the fact that combination of *sup45* mutations with [*PSI*$^+$] prion in diploids is fatal. Strong [*PSI*$^+$]^S variant, which is a strong suppressor, causes synthetic lethality in combination with all of the nonsense and some missense *sup45* mutations tested, while weak [*PSI*$^+$]^W or [*psi*$^-$] phenotypes do not lead to diploid cell death. The presence of extra copies of a gene that affects the manifestation of [*PSI*$^+$] or termination factors properties may lead to either increase or decrease in diploid lethality. To search for new genes that affect the translation termination efficiency and/or prion maintenance a gene library screen using the synthetic lethality test was performed. We identified several genes, including Q,N-rich transcription factor *MCM1*. We analyzed other known Q,N-rich transcription factors and discovered that the synthetic lethality also increases under overexpression of *GLN3*, *MOT3* and *SFP1*. We propose that Q,N-rich transcription factors may influence *SUP35* expression thus affecting [*PSI*$^+$]. This might represent novel pathway of prion-prion interactions, since 2 of the transcription factors are known prion determinants. The research was supported by RRC MCT SPbSU. The authors acknowledge Saint-Petersburg State University for a research grants 1.37.291.2015, 0.37.696.2013 and Russian Foundation of Basic Research for a research grants 13-04-00645 and 14-04-31265.

P.31: In vivo C2-fragmentation of the cellular prion protein in uninfected cells

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The domain organization of the cellular prion protein (PrP^C) consists of an unstructured N-terminal region, including a metal-binding octarepeat region (OR), a linker domain, and a C-terminal domain that misfolds to form PrP^{Sc} in diseases like Creutzfeldt-Jakob Disease. PrP^Ccan be alternatively processed; β-fragmentation that results in formation of C2 fragment, α-endoproteolysis and shedding. Also,

oligomeric A-Beta peptide has a major binding site between the alpha- and beta-sites of PrP^C.

β-cleavage of PrP^C has previously been suggested to occur through reactive oxygen species generated by the binding of copper to the PrP OR. We have recently reported generation of a novel *Prnp* allele, "S3" which has PrP^C OR conformationally-locked in a compact arrangement that favors binding of one copper ion per OR. Interestingly, this allele resulted in overproduction of C2 PrP in Tg.S3 mice and transfected RK13 cells, but not N2a and SMB cells. These observations, in conjunction with chelator studies, imply that b-processing of PrP^C may occur by a mechanism distinct from metal catalyzed hydrolysis reported *in vitro*. In this current study, using mass spectroscopy analysis of mouse brain S3 and Wt PrP^C, we have identified the "P1" amino acid residue for β-cleavage *in vivo*. This information, as well as studies with protease inhibitors, has narrowed the possible identities for a β-site protease. As alpha- versus beta-processing of PrP^C can lead to benign outcomes in the presence of PrP^{Sc} or oligomeric A-Beta, our findings may provide useful insights into the pathogenesis of both prion and Alzheimer's disease.

P.32: A biochemical approach to evaluate the contribution to pathological prion protein formation from the 222K PrP variant in scrapie positive goats

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Lysine (K) at position 222 of goat PrP is associated with resistance to classical scrapie, yet few natural cases of scrapie have been found in Q/K goats and intracerebral experimental transmission was successful in Q/K and K/K goats after long incubation periods. Previous Western Blot (WB) studies performed by different monoclonal antibodies (mAbs) revealed an inhibition by K222 on the binding of mAb F99/97.6.1 to goat PrP. Thus a WB method, based on the ratio between the signal intensity given by mAbs F99/97.6.1 and SAF84, was developed to distinguish goats exhibiting PrP encoded by the genotypes 222Q/Q, 222Q/K and 222K/K. Here we applied this approach to investigate the contribution to PrP^{Sc} formation given by the 222K variant in scrapie positive goats. WB analyses were performed on the PrP^C or PrP^{SC} extracted from the brains of negative and scrapie positive goat samples (natural or experimental), bearing different genotypes at position 222. Samples were analyzed in triplicate and signals revealed by F99/97.6.1 and SAF84 were quantified. A descriptive statistical analysis was performed.

No signals were detected by F99/97.6.1 in any of the 222K/K goat samples. The ratio of the optical densities revealed that the positive 222Q/K goats had a similar reactivity to 222Q/Q samples. Halved values of the ratio were present in negative goats with 222Q/K. The statistical analysis confirmed these differences. The obtained results showed that in heterozygous animals PrP^{Sc} seems to be formed nearly totally by the Q222 variant thus confirming the higher resistance to convertibility of K222 PrP variant.

P.33: Genetic modulation of atypical scrapie transmissions in transgenic mouse models

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Atypical scrapie, a recently recognized and surprisingly prevalent prion disease of sheep, is an example of an emerging prion disease of uncertain origin and species tropism. Since its discovery in 1998, precautionary testing have revealed surprising numbers of atypical scrapie cases in Europe. In 2007, a sheep from a flock in Wyoming tested positive for atypical scrapie. Atypical and classical scrapie differ with biochemical features of the protease-resistant form of the prion protein (PrP^{Sc}), and neuropathology. Also, atypical scrapie occurs in sheep with *PRNP* genotypes usually associated with resistance to classical scrapie, and sheep with atypical scrapie are more likely to express phenylalanine (F) than leucine (L) at codon 141. The origin and host range of atypical scrapie are unclear. To address these issues, we generated transgenic mice expressing Ovine PrP with phenylalanine at the residue 141, Tg (OvPrP-F141). We produced 2 sublines: the brains of the Tg(OvPrP-F141)H express PrP^{C} ~5-fold higher than wild type mice, while Tg (OvPrP-F141)L mice express at ~2-fold. Tg (OvPrP-F141)H spontaneously developed truncal ataxia and limb paresis at a mean age of 70 d. Remarkably, PrP^{Sc} accumulated in brains and muscles of sick mice. Furthermore, we inoculated the Tg(OvPrP-F141)L, Tg(OvPrP-A136), and Tg(OvPrP-V136) mice with 6 atypical scrapie isolates to determine the effects of the F141 genotype on atypical scrapie propagation. Only Tg(OvPrP-F141)L developed clinical signs and the brain samples showed typical biochemical signature of atypical scrapie. Our findings suggest that atypical scrapie may have spontaneously originated and subsequently propagated and evolved by transmission in sheep with susceptible genotypes.

P.34: Preliminary study of Alzheimer's disease transmission to bank vole

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Extensive experimental findings indicate that prion-like mechanisms underly the pathogenesis of Alzheimer disease (AD). Transgenic mice have been pivotal for investigating prion-like mechanisms in AD, still these models have not been able so far to recapitulate the complex clinical-pathological features of AD. Here we aimed at investigating the potential of bank vole, a wild-type rodent highly susceptible to prions, in reproducing AD pathology upon experimental inoculation.

Voles were intracerebrally inoculated with brain homogenate from a familial AD patient. Animals were examined for the appearance of neurological signs until the end of experiment (800 d post-inoculation, d.p.i.). Brains were studied by immunohistochemistry for pTau

(with AT180 and PHF-1 antibodies) and β-amyloid (4G8).

Voles didn't show an overt clinical signs, still most of them (11/16) were found pTau positive when culled for intercurrent disease or at the end of experiment. Interestingly, voles culled as early as 125 d.p.i. already showed pTau aggregates. Deposition of pTau was similar in all voles and was characterized by neuropil threads and coiled bodies in the alveus, and by rare neurofibrillary tangles in gray matter.

Conversely, β-amyloid deposition was rather rare (2/16). Nonetheless, a single vole showed the contemporaneous presence of pTau in the alveus and a few Aβ plaque-like deposits in the subiculum. Uninfected age-matched voles were negative for pTau and Aβ.

These findings corroborate and extend previous evidences on the transmissibility of pTau and Aβ aggregation. Furthermore, the observation of a vole with contemporaneous propagation of pTau and Aβ is intriguing and deserves further studies.

P.35: Assessing milk from CWD-lactating deer for infectious prions

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Transmissible spongiform encephalopathies (TSEs), or prions, cause a fatal neurodegenerative disease in mammals including bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep, variant Creutzfeldt-Jakob disease in humans and chronic wasting disease (CWD) in deer, elk and moose. CWD, the only prion disease to infect a native free-ranging population, has now been detected in 23 US states, 2 Canadian provinces and South Korea. While horizontal transmission is credited for much of the spread of CWD, few studies have monitored the potential for vertical/maternal transmission with an emphasis on lactation. Using a small, polyestrous cervid—the Reeves' muntjac deer—we are addressing this issue by supplementing naïve Reeve's muntjac fawns (n = 5) with previously collected muntjac milk. Each fawn (n = 3) was orally dosed with pooled muntjac colostrum (6mls colostrum at 24 hours) and milk (6mls milk per day for an additional 15 d [total of 90mls]) from pre-clinical and clinical CWD+ doe. Negative control fawns (n = 2) received similar colostrum/milk harvested from naïve doe. CWD status of inoculated fawns and their dams will be monitored by immunohistochemistry, real time quaking induced conversion assay (RT-QuIC), protein misfolding cyclic amplification (PMCA) and clinical disease progression. The results of this study will establish: 1) if there are sufficient infectious prions in the milk of lactating doe to transmit disease to offspring and 2) if mother to offspring transmission plays a role in the high efficiency with which CWD is transmitted in nature.

P.36: Spontaneous in vitro conversion of full length recombinant human prion protein in unseeded RT-QuIC reactions

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Sporadic Creutzfeldt–Jakob disease (sCJD) is the most common human prion disease, affecting approximately 1–2 persons per one million of the population per year. It is thought to arise as a result of spontaneous conversion of PrP^{C} to PrP^{Sc}, which becomes self-propagating. The prion protein polymorphism at codon 129 encodes either methionine (M) or valine (V). Comparison of the codon 129 genotype distribution in sCJD cohorts with that of the normal Caucasian population suggests that heterozygosity (MV) protects against sCJD and the comparison has also been widely interpreted to mean that methionine homozygosity predisposes to CJD.

We have used real-time quaking induced conversion (RT-QuIC) to model the

spontaneous formation of the abnormal form of human PrP and to determine whether methionine or valine at the position 129 of PrP^C confers a greater susceptibility to spontaneous conversion to PrP amyloid.

Unseeded RT-QuIC reactions using full-length recombinant human prion protein with either methionine or valine at position 129 both resulted in spontaneous amyloid formation. The process appeared to have a pronounced stochastic element, but when a sufficient number of replicates were performed a clear and reproducible effect of codon 129 genotype was also evident, in which PrP^C with valine at codon 129 showed a greater predisposition to form amyloid than its allelic counterpart containing methionine.

These results question whether methionine at position 129 in PrP^C can be considered an intrinsic susceptibility factor for conversion to PrP^{Sc}, at least in terms of the initiation of spontaneous, as opposed to seeded PrP amyloid formation.

P.37: The differential distribution of actin-cofilin rods in the brains of prion inoculated transgenic mice

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The neuropathology of transmissible spongiform encephalopathies (TSEs) includes the loss of synapses and neurons within the central nervous system (CNS). Potential causes of this CNS pathology include glutamate toxicity and oxidative stress, which have also been shown to induce ADF/cofilin rods within neuronal processes in both cultured hippocampal neurons and Alzheimer's disease brain. Cofilin rods can completely occlude neurites leading to loss of function distal to the rods. In this study, we are interested in determining if there are increases in the presence of ADF/cofilin rods in prion-inoculated transgenic mouse brains leading to the loss of neuronal processes and ultimately neurons as the disease progresses. Tg5037 mice, expressing cervid PrP (prion protein), and TgA20 mice, overexpressing mouse PrP, were ic inoculated with E2 (CWD elk brain homogenate) and RML (Rocky Mountain Labs mouse adapted scrapie) respectively. Controls included Tg5037 and TgA20 mice inoculated with normal brain homogenate (NBH) and FVB and PrP% mice inoculated with E2 or RML. TgA20 mice were euthanized at 40dpi, 50dpi, and when clinical signs appeared; Tg5037 mice were euthanized at 80dpi, 120dpi, and when terminal. Brains were removed, fixed, frozen or wax embedded, sectioned and examined by immunostaining for the presence of cofilin rods and PrP^{Sc}. In Tg5037 mice, rods occurred predominantly in the inferior olivary complex in the brainstem. In TgA20 mice, rods began to appear at 40dpi in the superficial cortex, and spread to deeper areas of the cortex and other regions of the brain by 50dpi.

P.38: Deletion of the C-terminal part of prion protein (PrP^C) leads to ER retention, p38 MAPK activation and neurodegeneration in transgenic mice

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Many aspects of PrP^C biology and conversion to PrP^{Sc} are still enigmatic. Transgenic mice lacking PrP^C ($Prnp^{0/0}$) do not display any gross disease phenotype that could shed light into the mechanisms of neurodegeneration. However, some transgenic mice with deletions at the N-terminal and central domains or insertions and point mutations in PrP^C, present a clinical phenotype reminiscent of prion disease. Interestingly, several mutations associated with human prion diseases are clustered in the C-terminal part of PrP^C, which probably affects PrP^C stability and leads to the misfolding of its

structured globular part. Point mutations located here also show transmission properties in transgenic mice.

We have studied a deletion mutant of PrP^C lacking 16 C-terminal amino acids (PrPD214–229) between the disulfide bond (from Cys178 to Cys213) and the omega site for GPI-anchor attachment (Ser230). In cells, we observed that this deletion interferes with the maturation through the secretory pathway as PrPD214–229 and it is retained in the ER. Remarkably, mice expressing PrPD214–229 present with a neurological disease showing a different clinical presentation compared to classical prion disease. In these mice, PrPD214–229 is also partially retained in the ER and PK resistant. Importantly, we show a clear activation of p38 MAPK both at pre-clinical and clinical time points. To our knowledge, this is the first time that aberrant trafficking of PrP is associated with a specific signaling pathway leading to neurodegeneration and a neurological disease in mice.

P.39: Essential collective dynamics analysis of unfolded α-synuclein dimers

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The collective motions of group of atoms in a protein influence its folding and stability, and may help identify early stages of protein misfolding. In this study, we looked into the folding dynamics of α-synuclein protein dimers. α-Synuclein is localized in the presynaptic nerve terminals found mainly in the brain tissue. Its misfolding is linked to certain neurological disorders like Parkinson disease. We investigated the folding of α-synuclein via all-atom molecular dynamics (MD) simulations starting with unfolded dimer models without any secondary structures. The resulting MD trajectories were analyzed using both conventional analysis of secondary structures and essential collective dynamics (ECD) - a novel methodology for identification of dynamic structural domains of correlated motions; analysis of the local flexibility of proteins; and construction of correlation maps that uncovers the existence of networks of dynamically correlated groups of atoms. In the course of the MD simulations, we found that the initially unfolded α-synuclein dimers collapse but retain most of their local alignment. After about 5–10 ns, they developed pronounced parallel or anti-parallel β-sheets. The arising β-sheets often belong to single ECD dynamical domains indicative of relatively rigid structures moving coherently. When β-sheets are formed involving 2 adjacent chains of the dimer, the ECD flexibilty profiles of the main-chain atoms tend to show similar levels of flexibility for both chains confirming a build-up of rather uniform dynamics in these regions. Pair correlation maps suggest that immediate interatomic contacts, rather than distant interactions, appear to determine the dynamics trends in the dimers.

P.40: Prion strain interference is a common property of prions

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Prion strain interference involves one strain blocking the emergence of a second strain and may be involved in prion adaptation. The long incubation period drowsy (DY) strain of transmissible mink encephalopathy (TME) can interfere with several short incubation period strains by many different routes of infection. It is unknown if other long incubation period strains can interfere with short incubation period strains. To explore this, we utilized the long incubation period 139H strain of hamster-adapted scrapie as the blocking strain. Sciatic nerve inoculation of 139H 25, 50 or 75 d prior to superinfection with the short incubation period hyper (HY) TME strain resulted in the 25-day and 50-day interval groups having incubation periods and clinical signs similar to HY

TME. Conversely, 4 of the 5 animals in the 75-day interval group had 139H clinical signs and incubation period. Clinical diagnosis was confirmed based on the strain-specific PrP^{Sc} conformational stability profiles. Based upon these results, 139H was able to block the emergence of HY TME in the 75-day interval group, indicating that strain interference had occurred. We then determined that sciatic nerve inoculation of 139H results in transport of PrP^{Sc} along the same 4 descending motor tracks as HY and DY TME indicating that 139H strain interference occurs in ventral motor neurons of the lumbar spinal cord. Overall, these studies indicate that prion strain interference is a common property of prions and has implications for the maintenance and evolution of prion strains.

P.41: Sporadic distribution of prion-forming ability of Sup35p and Ure2p from yeasts and fungi

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The Ure2p and Sup35p are 2 of several proteins capable of forming infectious amyloid in the yeast Saccharomyces cerevisiae. Ure2p functions in the ability of the cell to respond to the quantity and quality of nitrogen sources available in the growth environment and can form the [URE3] prion. Sup35p is part of the translation termination machinery and can form the [PSI+] prion. For both proteins their functionality is compromised upon prion formation. Both proteins also show remarkable evolutionary conservation of their domain structures. The domains of Ure2p and Sup35p that form amyloid are devoid of defined secondary structure. Nevertheless these domains are functionally important: binding microtubules and controlling mRNA turnover in Sup35p and stabilizing Ure2p. Homologues of Sup35p and Ure2p from other yeasts have been shown to be capable of prion formation implying that prion-forming ability of these proteins is conserved in evolution and thus has survival/fitness value for these organisms. The presence of either prion can have a substantial negative effect on the cells, especially de novo generated prions. Even the mildest variants of these prions are rare in wild isolates. We surveyed a number of yeast and fungal species for the ability of their Sup35 or Ure2p homologues to form prions in S. cerevisae. We find that many of the assayed proteins could not form prions suggesting that prion-forming ability is not a conserved trait but is a side effect of a domain conserved for another function.

P.42: PrP^{Sc}-containing brain samples induce PrP^{C}-dependent retraction of dendritic spines in primary neurons

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Despite intensive investigation, how PrP^{Sc} induces neuronal degeneration is still unknown. Synaptic loss is an early event in prion disease, based on immunostaining of infected brain slices (Mallucci et al. Neuron 53, pp325–335 2007). However, the cellular and molecular mechanisms underlying this process have been difficult to study, primarily because of the absence of systems for modeling PrP^{Sc}-induced synaptotoxicity *in vitro*. In our lab, we have been attempting to develop such a model system. Thus far, we have now shown that PrP^{Sc}-containing brain homogenates from infected mice induce dendritic spine retraction and synaptic loss in mature mouse hippocampal neurons. In contrast, brain homogenate from normal brain does not induce synaptic loss. Further, we observed that the effect is PrP^{C}-dependent, as PrP^{Sc}-containing brain homogenate does not induce synaptic loss in neurons from PrP knockout mice. Interestingly, the synaptotoxic effect requires the N-terminal domain of PrP^{C}. When neurons cultured from transgenic mice expressing an N-terminally deleted form of PrP^{C} (Δ23–111) were treated with infected brain homogenate, no synaptic loss was observed. These results establish a cell

culture system for analyzing how PrP^{Sc} affects neuronal function and synaptic integrity.

P.43: Cell culture models for studying CWD prions

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Chronic wasting disease (CWD) of cervids is the most contagious prion disease. CWD prion infectivity is found in many tissues, body fluids and secretions. Cell culture models provide the opportunity to study the cellular and molecular biology of prion infections. Mostly scrapie-derived and mouse-adapted prions have been studied in cell models. Currently, only modified RK13 cells are available as persistently infected model for CWD prions. We want to develop new cell culture models for acute and persistent propagation of CWD prions. Our rational is to use cell lines derived from cervid tissue which endogenously express cervid PrP^{c}. We have established skin-derived stem cells from antler tissue of Caribou as such stem cells are multipotent and allow differentiation into other lineages. Importantly, prion infection of antler velvet was found in vivo. In addition, we use a non-transformed fibroblast cell line from Indian Muntjac deer, as fibroblasts are known to be well susceptible to prion infection. Using immunoblot and confocal microscopy analysis we found that both cell lines express normally glycosylated PrP^{c} and that PrP^{c} is located at the outer leaflet of the plasma membrane. We are now in the process of infecting these cells with CWD prions derived from brains of transgenic mice or deer and elk. PrP^{Sc} uptake and prion propagation is studied using immunoblot and confocal microscopy read-outs. Novel CWD prion propagating cells will be useful for elucidating the molecular mechanisms underlying the pronounced lateral spread of CWD prions.

P.44: The influence of proteinase-activated receptor 2 deficiency on the course of prion infection: An experimental study using murine model

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Background. Proteinase-activated receptors (PARs) play an important role in modulation of many pathological processes including neurodegeneration. Recently, we have demonstrated that deletion of trypsin receptor PAR-2 prolongs survival of mice intracerebrally inoculated with RML prions.[1] PAR-2 is expressed in many cells and tissues including immune organs which are involved in peripheral prion propagation. The aim of our study is to evaluate the effect of PAR-2 deletion on survival of mice after peripheral inoculation with prions.

Methods. PAR-$2^{+/-}$ heterozygous mice were bred to obtain littermates sharing the same genetic background except for PAR-2 expression (PAR-$2^{-/-}$, PAR-$2^{+/-}$, PAR-$2^{+/+}$). 48 sibling mice were inoculated subcutaneously with high dose of RML prions. To prevent the influence of subjective factors, the experiment is blind and mice were not genotyped. Mice are observed for signs of scrapie and their weight is monitored. Mice in terminal stage of the disease are euthanized and organs are harvested for genotyping and further analyses.

Results. To date, 27 out of 48 infected mice were euthanized. Mean survival and number of animals in the genotype groups are as follows: PAR-$2^{+/+}$ (232.5 ± 6.8 d, $n = 6$); PAR-$2^{+/-}$ (232.6 ± 9.8 d, $n = 16$); PAR-$2^{-/-}$ (237.0 ± 7.6 d, $n = 5$). All remaining infected mice are symptomatic.

Conclusions. Our preliminary data suggest that the survival of mice after peripheral inoculation with prions does not seem to be notably affected by PAR-2 deletion. Final data will be presented at the meeting.

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Reference

1 Matej R, et al. J Gen Virol 2012; 93:2057–61; PMID:22694901; http://dx.doi.org/10.1099/vir.0.043877-0

P.45: Peptide aptamers binding to PrPC inhibit prion protein misfolding

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Currently neither therapeutic nor prophylactic tools are available for treatment of prion diseases. In order to identify anti-prion compounds, we aim to inhibit prion conversion by interfering with the interaction between the cellular prion protein PrPC and its infectious form PrPSc, which represents the first step in the pathogenesis of these fatal disorders.We have previously described a peptide aptamer (PA8) binding to PrPC which reduces prion propagation in prion infected cultured cells. Peptide aptamers consist of combinatorial peptides inserted into a scaffold protein. To improve binding affinity, and thereby the anti-prion activity of PA8, its interaction with PrPC has been modeled in silico. Three residues of the 16mer peptide were defined as targets for amino acid substitutions that are expected to strengthen the PA8-PrPC interaction. According to these results, we have created selected PA8 variants by introducing single amino acid exchanges in the peptide moiety. All PA8 variants were expressed in E. coli, purified via His6-Tag and employed for treatment of prion-infected neuroblastoma cells. Using different concentrations of purified peptide aptamers, we identified three variants that have shown an enhanced inhibition of PrPSc conversion in comparison to the original PA8. We plan to evaluate their activity also by transfection of persistently scrapie infected cells with PA8 variants that harbor cellular targeting signals and in mouse bioassays.In future, our results will be useful for identifying a compound by rational drug design based on the structure of the PA-PrPC complex which is able to counteract prion conversion in vivo.

P.46: Mitigation of prion infectivity and conversion capacity by a simulated natural process—repeated cycles of drying and wetting

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Prions enter the environment from infected hosts, bind to a wide range of soil and soil minerals, and remain highly infectious. Environmental sources of prions almost certainly contribute to the transmission of chronic wasting disease in cervids and scrapie in sheep and goats. While much is known about the introduction of prions into the environment and their interaction with soil, relatively little is known about prion degradation and inactivation by natural environmental processes. In this study, we examined the effect of repeated cycles of drying and wetting on prion fitness and determined that 10 cycles of repeated drying and wetting could reduce PrP^{Sc} abundance, PMCA amplification efficiency and extend the incubation period of disease. Importantly, prions bound to soil were more susceptible

to inactivation by repeated cycles of drying and wetting compared to unbound prions, a result which may be due to conformational changes in soil-bound PrP^{Sc} or consolidation of the bonding between PrP^{Sc} and soil. This novel finding demonstrates that naturally-occurring environmental process can degrade prions.

P.47: An apparently universal substrate for RT-QuIC-based detection and discrimination of prion strains

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Prions propagate as multiple strains and can be found in an assortment of sub-types in many mammalian species. The detection of all such prion populations by a single ultrasensitive assay would be valuable in prion disease diagnosis, surveillance and research. Here we show that under novel conditions a previously untested recombinant prion protein is an effective substrate for the sensitive RT-QuIC detection of a total of 26 prion types/strains tested thus far from humans, cattle, sheep, cervids and rodents. These prion strains include several that were previously undetectable, such as sheep Nor98 scrapie and some human GSS prion seeds. Furthermore the ability of different prion seeds to induce polymerization of specific RT-QuIC substrates can be exploited to discriminate prion strains such as sporadic and variant Creutzfeldt-Jakob disease in humans, classical and atypical Nor98 scrapie strains in sheep, and classical and atypical BSE in cattle. Moreover, striking strain-dependent differences in the protease-resistant banding profiles of the RT-QuIC conversion products was observed, suggesting the existence of multiple distinct classes of prion templates and additional means of strain discrimination.

P.48: Biochemical properties of chronic wasting disease prions and relationship with prion shedding

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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 still remains. In most prion diseases, abnormal prion protein (PrP^{Sc}) is mainly found in the brain. However, in CWD (alike scrapie), PrP^{Sc} is detectable in many extraneural tissues, which contributes to the rapid spread of the disease. Studies suggest a link between biochemical stability of strains, incubation time of disease and neuroinvasive properties. These data led to our hypothesis that prion transport and shedding depend on intrinsic biochemical properties of strains. Thus, we analyze the PrP^{Sc} aggregate composition, proteinase K (PK) resistance and conformational stability of CWD-elk, -deer, -white-tailed deer and -mule deer, and ME7 scrapie prions. Our results suggest similar PK-resistance of CWD and scrapie strains. However, the conformational stability ($GdnHCl_{1/2}$) of these prion strains is different. Moreover, velocity sedimentation fractions of CWD and

scarpie strains show a different distribution. PrP^{Sc} from CWD-elk is found in fractions with a lower density. These results suggest a different aggregate-stability and -size distribution. We aim to elucidate whether CWD prions have certain properties that make them more prone to be transported from the brain to peripheral organs. Our results will add novel knowledge about pathogenesis of CWD and might elucidate targets that help to prevent prion shedding which may help further spread of CWD and thus, reduces a potential future risk to human and animals (cervids and non-cervids).

P.49: 2-cysteine-substitution mutants of PrP for studying the interfaces of PrP^{C}-PrP^{Sc} interaction

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We introduced 2-cysteine-substitutions (C; C-PrP) into PrP, one at a residue in the region between helix 1 and 2 (H1∼H2) and the other one between residues 220 and 229 (Ctrm), to form an additional disulfide bond and to introduce structural constraints into PrPc. Interestingly, depending on positions of the cysteine in Ctrm, such PrPs were converted into PrP^{Sc} in persistently prion-infected cells. We found that C;C-PrPs with 165C-229C, 166C-229C, and 168C-225C showed substantial levels of PK-resistant fragments. These findings implied that the H1∼H2 portion can undergo a positional change toward Ctrm during interaction with PrP^{Sc}. Next, we assessed influences of disulfide crosslinks on dominant-negative inhibition (DNI). We introduced a deletion of residue 159 (Δ159) to C;C-PrP constructs (ΔC;C-PrP) and observed their DNI in 22L-infected N2a cells. DNIs of ΔC;C-PrPs were highly affected by positioning of the cysteine in Ctrm: some C;C-PrPs substantially lost DNI by combination with Δ159, while others exerted efficient DNI. These results imply that the aforementioned positional change of H1∼H2 and the subcellular localization of PrPc constructs influence how PrP^{C} interacts with PrP^{Sc}. It also suggests that, when affinity of one interface is weak, another interface might take over as a main interface for PrP^{C}-PrP^{Sc} interaction. Such a shift of main interface might occur during heterologous PrP^{C}-PrP^{Sc} interaction in interspecies transmission or depending on microenvironments and cofactors. Thus, studies with C;C-PrPs will help to elucidate the molecular and cellular mechanisms in the interaction of PrP^{C} with PrP^{Sc} which is a prerequisite in prion conversion.

P.50: Assessment of high pressure digestion prion mitigation by bioassay

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Approximately 150 million people and almost $40 billion worth of agricultural commodities pass through US international ports annually. Ports seize animal and plant products potentially contaminated with high risk diseases that then must be decontaminated. In this study we assess the efficacy of alkaline digestion to mitigate infectious prions. Transmissible spongiform encephalopathies (TSEs), or prions, were chosen as the infectious agent for this study because they are difficult to inactivate, affect both human and animal species worldwide and are shed by infected hosts into the environment establishing highly infectious biota. Chronic wasting disease (CWD), the TSE of cervid species in North America, has recently been spotlighted as a potential concern for European countries, and recapitulates human and animal TSE pathogenesis. We processed CWD positive and negative brain tissue by alkaline digestion under standard temperature and pressure at time intervals of 2, 4, and 6 hours. Digested tissues were analyzed for residual infectivity by bioassay into transgenic mice expressing the cervid prion protein (CerTg5037) and amplification competent prions by in vitro RT-QuIC methodology. While bioassays are ongoing (330 dpi), preliminary results indicate that prion contaminated

tissues may be rendered noninfectious after a single 4 hour cycle of alkaline digestion. This work will provide a basis for future studies designed to unravel mechanisms associated with the ability of prions to bind surfaces, enhancing prion mitigation strategies.

P.51: Targeted zebrafish mutants to unveil normal functions of, and interactions between, prion protein and amyloid precursor protein: Relevance to Alzheimer disease

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Objective. We aim to uncover the elusive functions of prion protein (PrP) and amyloid precursor protein (APP) and to identify therapeutic targets for prion diseases and Alzheimer disease. Zebrafish have 2 homologs of PRNP (prp1 and prp2) and APP (appa and appb) that have functional conservation with their mammalian counterparts. Our demonstration of a genetic interaction between prp1 and appa supports novel roles for PrP in Alzheimer disease (Kaiser et al. 2012 PMID: 23236467).

Methods. We recently created and characterized zebrafish with a null prp2 allele (Fleisch et al. 2013 PMID: 23523635). Here we measured c-fos expression by in situ hybridization and qPCR as a molecular indicator of neuron activity in zebrafish exposed to the convulsant pentylenetetrazole (PTZ). We used TAL Effector Nucleases (TALENs) to mutagenize zebrafish prp1 and appa.

Results. PTZ treatment induced significantly more c-fos expression in 2 dpf prp2−/− larvae relative to wild type. We also engineered novel zebrafish with TALEN-induced frameshift mutations in appa and prp1. Assessing phenotypes in zygotic prp1−/−, appa−/− and compound mutants is underway.

Discussion. Enhanced expression of c-fos in 2 dpf prp2−/− larvae compared to wild type larvae after PTZ exposure further supports a protective role for PrP2 against neuronal hyperexcitability. This phenotype provides a platform to map neuroprotective functions to specific PrP protein domains via mRNA rescue experiments. Our novel Prp1 and appa mutants will be bred to study interactions between PrP and APP paralogs. Our findings have important implications for design of prion disease and Alzheimer disease therapeutics.

P.52: Pentosan polysulfate initiates prion fibrillization by inducing intramolecular, hydrophobic interactions of the octarepeat with the C-terminus

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We used thioflavin (ThT) fluorescence, intrinsic tryptophanyl fluorescence (ITF) and circular dichroism (CD) spectroscopy to measure hydrophobic transitions in the solvent environment of the PrP octarepeat (OR) tryptophanyl side chain cluster (OR_{tc}) during fibril formation induced by the synthetic heparinoid pentosanpolysulfate (PPS). By studying spectral intensity and wavelength shifts in full length prion protein (PrP_{23-231}), N-terminally truncated material ($PrP_{112-231}$, PrP_{90-231}) and PrP octarepeat peptide (OR, PrP_{53-90}) we observed that the OR_{tc} is in a more hydrophobic environment in native PrP_{23-231} than in free OR peptide (PrP_{53-90}). This indicates an intermolecular hydrophobic burial of the OR_{tc} in PrP_{23-231}. PPS induced rapid fibril formation and an increase in ITF of PrP_{23-231} and PrP_{90-231}. Fibril formation by PrP_{90-231}indicates there is a C-terminal PPS-binding site.

We conclude that in PrP_{23-231}, the N-terminal OR_{tc} has intramolecular affinity for the C-terminal hydrophobic domain (HD, $PrP_{112-122}$) and when bound to PPS forms a tertiary, hydrophobic complex, which leads to fibrilization. We propose that 1) heparinoid molecules may induce a PrP oligomerization to a dodecamer which is biologically active; 2) a defective

heparan structure may result in induction of a stable pathological PrP fibril structure.

P.53: Active vaccination using multimeric PrP as immunogen as strategy to contain chronic wasting disease

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Chronic wasting disease (CWD) is the only prion disease which occurs in free ranging and captive animals. It expands geographically and in numbers in North America and the potential for transmission into humans cannot be excluded. Effective measures for containing CWD are therefore crucial. It is our central hypothesis here that it is feasible to interfere in peripheral prion infection and prion shedding by inducing auto-antibodies against PrPc 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, notably without adverse side effects. Auto-antibodies neutralized prion propagation in cultured cells and up to 25% of immunized and prion challenged mice survived prion infection. Having a reliable proof-of-concept for our strategy in wild-type mice we are now focusing on active vaccination against CWD. We expressed in E.coli, purified and refolded 4 immunogens: cervid and murine PrP in monomeric and dimeric form. As delivery strategy allowing oral vaccination we co-encapsulated recombinant PrPs with various adjuvants into microspheres. For testing immunogenicity and safety we are currently performing immunization studies in wild- type and cervid PrP-transgenic mice. Protection against CWD will be tested by infecting immunized mice with mouse and cervid prions. Our long-term goal is to implement a translational research program for developing a wild-life suitable vaccine which is able to reduce levels of morbidity and mortality and further transmission of CWD.

P.54: Infectious recombinant prions: In vitro generation and propagation of different strains

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

different recombinant prion strains with robust in vitro self-replicating abilities but dissimilar infectious features in vivo.

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

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

P.55: Discrimination of classical and atypical BSE by a distinct PrP^{Sc} profile

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Bovine spongiform encephalopathy (BSE) belongs to the transmissible spongiform encephalopathies and is associated with the accumulation of a pathological isoform of a host-encoded glycoprotein, prion protein (PrP^{Sc}). To date, classical BSE (C-type) and 2 atypical BSE forms (L- and H-type), are known and their discrimination is based on the biochemical characteristics. The challenge to surveillance programs is to differentiate classical BSE from atypical BSE cases, since C-type BSE is feed borne and atypical BSE still of unknown origin. However, only Western blot analysis allows a clear discrimination between the BSE forms until now. The goal of our study was to identify type-specific PrP^{Sc} profiles by using Immunohistochemistry as an additional method.

In total 21 cattle, intracerebrally inoculated with C-type, H-type and L-type BSE were used. From these animals, 6 well-defined brain areas were examined by H&E staining and by Immunohistochemistry (IHC), using 2 different C-terminal antibodies.

The histopathological examination revealed clear differences among the individual cattle, but the immunohistochemical data points toward the existence of a distinct PrP^{Sc} profile between the different BSE-types. This profile involved both the specific brain areas affected and the cellular pattern of the PrP^{Sc} deposition.

The qualitative and quantitative analysis of PrP^{Sc} affected structures shed new light into the pathogenesis of the different BSE types. Additionally, the results presented here support IHC characterization as additional diagnostic tools in classical and atypical BSE surveillance programs, in particular when only formalin fixed tissue samples are available.

P.56: Factors that improve RT-QuIC detection of prion seeding activity

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The Real-Time Quaking-Induced Conversion (RT-QuIC) assay for prion-associated seeding activity has been applied successfully to a wide variety of transmissible spongiform encephalopathies (TSEs) and tissues and is being implemented in ante mortem diagnostic testing. Our laboratory has continued to explore assay factors in order to assess and improve performance and practicality. Included in the many reaction parameters that we have evaluated are substrate selection, temperature, shaking conditions, pH and NaCl concentrations. Our results indicate that a high degree of sensitivity and specificity are achieved by optimizing these conditions within certain ranges. We demonstrate

that replacing full length recombinant prion protein ($rPrP^{Sen}$) substrate with N-terminally truncated $rPrP^{Sen}$ (residues 90–231) decreases the assay's lag time. Similar effects were observed, without compromising specificity, when either the reaction temperature or the shaking speed was increased. An optimization of concentrations between 130mM and 300mM NaCl provides specificity by reducing prion-independent amyloid formation. Furthermore, we find that pH 7.4 provides for more rapid and prion-specific RT-QuIC reactions compared to more acidic pH's. When a cumulative optimization of these conditions was applied to nasal brushings from human sporadic Creutzfeldt-Jakob disease patients, higher temperatures reduced RT-QuIC lag phases, and the use of hamster recombinant prion protein 90–231 as substrate strengthened prion-associated signals. Collectively, these results demonstrate improved speed, efficacy and practicality of RT-QuIC assays and highlight variables to be optimized for applications to new prion strains and sample types.

P.57: Examining the role of PrP^c in regulating neuronal activity and myelination

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Our research uses zebrafish to examine the function of PrP^c in an intact in vivo animal model. Previously our lab has shown altered NMDA receptor kinetics in the Mauthner cell of $PrP2^{-/-}$ larvae (Fleisch et al. 2013 PMID:23523635). We are developing a simple and reliable behavioral assay for evaluating the role of PrP^c in regulating synaptic activity by exploiting the Mauthner cell controlled touch evoked escape response (TEER) in larval zebrafish. Thus far, results show that $PrP2^{-/-}$ larvae take 55% longer to complete this response ($p = 0.002$). This assay, combined with microinjection of domain swapping PrP^c mRNA constructs, will allow us to dissect the relevant domains of PrP^c for regulating neuronal excitability. Altered axonal conductance, a phenotype observed in $PrP^{-/-}$ mice exhibiting demyelination in their PNS, may contribute to this longer duration TEER. Consequently, we have evaluated the condition of myelin in the posterior lateral line nerve of $PrP2^{-/-}$ zebrafish, a PNS nerve important for tactile sensation. Preliminarily, in aged $PrP2^{-/-}$ zebrafish, we do not observe a similar thinning of the myelin as was seen in mice, but myelin and axons do display significantly altered structure. To better examine the function of PrP2 in myelin maintenance we have been testing the ability of the antibody SAF84 to detect zebrafish PrP2. Thus far our testing suggests that SAF84 is indeed capable of detecting zebrafish PrP2. In summary, the data demonstrate that PrP^c knockout zebrafish are tractable models for examining the role of PrPc at both the behavioral and cellular level.

P.58: Recombinant mouse prion protein alone or combined with detoxified lipopolysaccharide affects colon immune protein levels of FVB/N mice

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Cellular prion protein (PrP^C) plays an important role in assembling tight junctions and barrier functions in the GI tract. The objective of this study was to evaluate whether recombinant moPrP alone or combined with detoxified LPS would affect concentrations of Slpi, Tlr4, CAMP, and Sprn in the colon under an *in vitro* Ussing chamber system. Colons were selected from 8 male FVB/N mice and were randomly assigned to 6 treatments: (1) pyrogen free H_2O on the mucosal side (CON), detoxified lipopolysaccharide on the mucosal side (D-LPS/M), mouse recombinant prion protein on the mucosal side (moPrP/M), detoxified LPS on the serosal side (D-LPS/S), moPrP and D-LPS on

mucosal side (moPrP + D-LPS/M), moPrP on mucosal side and D-LPS on serosal side (moPrP/M + D-LPS/S). After euthanization, colon tissue was immediately excised and mounted between the mucosal and serosal reservoirs of easymount Ussing diffusion chambers. After 40 min colon tissues were removed and stored at -80°C until analysis. Concentrations of proteins in colon samples were determined using commercially available ELISA kits. Results indicated that concentrations of Slpi in moPrP + D-LPS and moPrP/M + D-LPS/S treatment groups were greater than CON group. CAMP protein was decreased in the moPrP/S + D-LPS/S group as compared to CON samples, whereas concentrations of Tlr4 were decreased by D-LPS and PrP/M and PrP/M+D-LPS/M; and Sprn was lowered by D-LPS, PrP/M, and PrP/M+D-LPS but increased by PrP/M+D-LPS/S. Further research is needed to understand the importance of these protein modifications in the colon by moPrP and D-LPS.

P.59: Mouse recombinant prion protein alone or in combination with bacterial LPS modulated production of colon proteins involved in innate immune responses in FVB/N mice

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The objective of the present study was to test whether mouse cellular prion protein ($moPrP^C$) alone or in combination with lipopolysaccharide (LPS) would alter innate immunity proteins in the colon. Eight FVB/N male mice were used to collect colon samples, which were mounted into a Ussing chamber. Chambers on both sides of the Ussing chambers were filled with 7 mL of Krebs buffer. The mucosal side of the chambers were supplemented with 700 μL of pyrogen-free H_2O (CON). Treatment 1 consisted in adding 700 μL of a solution containing 100 μg/mL of lipopolysaccharide (LPS) from *E. coli* O111:B4 strain on the mucosal side of the chamber. Treatment 2 with 700 μL of a solution containing 100 μg/mL of recombinant mouse (mo)PrP^C (29–231) on mucosal side (PrPM). Treatment 3 contained $moPrP^C$ + LPS at the 100 μg/mL on the mucosal side of the colon (PrPM + LPSM). The last treatment contained $moPrP^C$ on the mucosal side and LPS on the serosal side of the chambers (PrPM + LPSS). Samples were collected at 40 min of the initiation of the experiment and CAMP, SLPI, TLR4, and SPRN in the colon were measured. CAMP was significantly greater in PrPM treatment compared to PrPM + LPSM. Concentration of SLPI were increased by PrPM, LPSM, PrPM, and LPSS compared to the CON group. Concentrations of TLR4 were lowered by PrPM, LPSM, and their combination vs CON. LPS on the serosal side lowered levels of TLR4. SPRN concentration was lower in PrPM and PrPM + LPSM.

P.60: New [*PSI*$^+$]-no-more mutation in *SUP35* with strong inhibitory effect on [*PSI*$^+$] propagation

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Prions can be defined as infectious or inherited protein agents which propagation is based on the ability to induce conformational conversion of cellular polypeptides. [*PSI*$^+$] factor is one of well-studied yeast prions. Previously we constructed 5 *SUP35* alleles coding the proteins with QQ or QN to KK substitutions within one of the oligopeptide repeats (Bondarev et al., 2013). Positions and type of mutation were chosen according to the model of superpleated β-structure proposed by Andrey Kajava. Two of such alleles led to [*PSI*$^+$] prion loss but by different molecular mechanisms. Recently we

obtained a new mutation within another oligopeptide repeat tht we not examined and probed its effect on prion stability. This allele, named *sup35-M0* according number of repeat, eliminated [PSI^+] very efficiently even in presence of *SUP35*. The prion loss reached up to 97% that exceeded the effect of *PNM2* (less 20%) and *sup35-M2* described previously (approximately 40%). Nevertheless overexpression of *sup35-M0* promoted [PSI^+] induction and the corresponding protein formed fibrils in vitro. Our data provide experimental proof of superpleated β-structure model with assumption about polymorphism of prion structures and underlying role of N-terminal region of Sup35p in [PSI^+] prion propagation.

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P.61: Investigating amyloid-β oligomer and fibril dynamics using a novel amyloid-β oligomer-specific antibody

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Oligomers of the amyloid-β (Aβ) peptide play key roles in neurotoxicity and region-to-region spreading of Alzheimer's disease (AD) neuropathology. We have produced a monoclonal antibody against a novel Aβ epitope that is exposed only when Aβ is in oligomeric form. The epitope consists of a constrained turn at residues 26–28: cyclized serine-asparagine-lysine (cSNK). We have shown, by dot blotting, atomic force microscopy, and electron microscopy, that our antibody (5E3) binds to synthetic Aβ oligomers, but does not recognize monomeric or fibrillar Aβ. Furthermore, 5E3 specifically recognizes Aβ oligomers in brains of AD patients and AD model mice. Our recent work demonstrates that 5E3 is a promising agent for immunotherapy to reduce toxic Aβ oligomers in the brain. However, we show here that 5E3 also provides a useful tool to investigate *in vitro* by electron microscopy the mechanisms and time course of Aβ oligomer and fibril formation. Additionally, we show that 5E3 can reduce *in vivo* propagation of Aβ pathology to the mouse cortex following seeding by injection into the hippocampus. This data is consistent with the hypothesis that Aβ oligomers displaying the cSNK epitope constitute the seed species of AD pathology. 5E3 can also be used as a tool to investigate the mechanism of seed propagation: we find that the Aβ oligomers displayed on the surface of extracellular vesicles from AD mouse brains, which may participate in propagation, expose the cSNK epitope. These investigations show that 5E3 is a promising reagent for both therapy development and basic mechanism inquiry.

P.62: Combining anti-prion compounds with complementary mode of action for achieving additive effects in vitro and in vivo

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The quest for therapeutic strategies in prion diseases did not yet result in drug regimens which are effective when used in prion-infected human beings. This may surprise as a huge variety of chemical compounds have been reported as showing rather promising anti-prion activities *in vitro* and to, although much less, extent also in animal models. Possible explanations might be that most compounds fail to cross the blood-brain-barrier at therapeutic concentrations and that increasing the blood concentration usually results in severe side effects. Successful animal models often use experimental paradigms which are based on post-exposure and extra-CNS effects, and therefore do not reflect the real situation in human therapy. Our rational is to use a combination of chemical compounds which showed certain anti-prion effects already *in vitro* and *in vivo* when used alone and from which the underlying molecular

mechanisms are well characterized. Given this, we can combine drugs with complementary molecular targets and potentially additive effects. Presently, we test drugs which target prion biogenesis (e.g. targeting PrP^c substrate) or prion clearance (e.g., autophagy inducing drugs) in prion-infected cultured cells at non-toxic concentrations. In parallel, selected combinations are used in mouse models. Using such a strategy we want to achieve additive or even synergistic anti-prion effects, allowing us to lower the needed concentrations and associated side effects in vivo. More refined future combination therapies might help to bridge the obvious gap between *in vitro* and *in vivo* results and provide a foundation for effective drug therapies against prion diseases.

P.63: Analysis of activation state of astrocytes with the progression of prion diseases

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Activation of astrocytes and microglia is one of the hallmarks in prion diseases. Data on the involvement of microglia in pathogenesis of prion diseases are accumulating; however the role of astrocytes in prion diseases is not well understood. To elucidate the involvement of astrocytes in pathogenesis of prion diseases, we analyzed activation states of astrocytes in prion-infected mouse brains. We established the method for isolation of astrocytes from mouse brains using magnetic activated cell sorting (MACS) with Anti-Astrocyte Cell Surface Antigen-2 (ACSA-2) antibody. Purity of astrocytes was analyzed by flow cytometric analysis and TaqMan assay. Eleven genes of cytokines, chemokines, neurotrophic factors and their receptors that are reported to be up-regulated in prion diseases and other neurodegenerative diseases were analyzed by TaqMan gene expression assay. Flow cytometric analysis showed that the ACSA-2-bound fraction contained approximately 95% of ACSA-2-positive cells and that contamination of CD11b-positive microglia and CD31-positive endothelial cells was less than 0.5%. ACSA-2-positive cells included GFAP (astrocytes marker)-positive and negative cells, whereas ACSA-2-negative cells included only GFAP-negative cells. This suggests the utility of ACSA-2 for astrocytes isolation. High gene expression of *GFAP* but a trace of *CD11b* and *CD31* gene expressions also demonstrated the successive isolation of astrocytes by anti-ACSA-2 antibody. Among genes tested, *CXCL10* chemokine gene expression was upregulated from 90 dpi to 120 dpi, whereas expression of remaining 10 genes showed no remarkable differences. The temporal up-regulation of *CXCL10* gene suggests that activation state of astrocytes changes with the disease progression.

P.64: Behavior of antiprion small molecules in RT-QuIC

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The *in vitro* prion seeding reaction known as real-time quaking induced conversion (RT-QuIC) has proven its utility as a highly sensitive and specific diagnostic tool [1]. Initial efforts have been made to explore RT-QuIC's potential to inform drug discovery [2,3], but the relationship between observed antiprion activity in RT-QuIC and potential for efficacy *in vivo* remains inconclusive. We systematically query this relationship by leveraging compounds with established antiprion activity *in vivo*. To date, though no small molecules have yet been reported as effective against a human prion strain [4,5], 3 orally bioavailable small molecules have been reported to dramatically extend survival time in mice infected with RML prions: IND24, cpd-b and anle138b [4,6,7]. These and many other compounds are

active in phenotypic assays, reducing the formation of proteinase K-resistant PrP^{Sc} in RML-infected cultured cells, but the lack of human prion-infected cell lines precludes application of these assays to human prion strains. Here we assess the ability of RT-QuIC to detect the established antiprion activity of these compounds *in vitro*. This approach could help to decipher the still elusive mechanism of action of these compounds, and if successful could be extended to testing candidate compounds against human prion strains.

P.65: Myotube cultures as a prion source for structural investigations

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Our knowledge about the prion diseases contains important gaps that need to be filled: E.g. the structure of the infectious isoform of the prion protein (PrPSc) and the conversion process from its cellular precursor (PrPC). Most techniques to decipher the structure of PrPSc are hampered by its insolubility and the need for substantial amounts of highly purified protein. Nearly all approaches that address the structure of PrPSc rely on brain samples from infected animals. In our current study we are trying to overcome the limitations of this source by using prion-infected C2C12 myotubes. Myotubes are non-proliferative cells that, upon infection with RML prions, can generate high levels of PrPSc and infectivity, about 10 times more than other mammalian cells in culture (e.g., N2a cells) (Herbst et al., 2013; PLoS Pathogens; 9:e1003755). We encountered difficulties in the purification of myotube-derived PrPSc via precipitation with sodium phosphotungstate due to the co-precipitation of fibronectin, fibrillin, and collagen; these extracellular matrix proteins were identified by mass spectrometry and validated through Western blotting. In OptiPrep gradients (sedimentation velocity and sedimentation equilibrium gradients), these proteins co-migrated not only with some fractions of myotube-derived PrPSc, but also with PrPC. Fortunately, fractionation of the OptiPrep gradients achieved a complete separation of PrPSc from the extracellular matrix proteins in many fractions, and provided additional information about the density and hydrodynamic properties of the PrPSc aggregates. Electron microscopy will be used to evaluate the quaternary structure of the PrPSc aggregates and their overall suitability for structural studies.

P.66: Transport of CWD prions in Alberta soils

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The transmission of chronic wasting disease (CWD) includes environmental pathways, particularly soils as disease reservoirs. Soils differ dramatically in their capacity to adsorb PrP^{CWD} due to differences in mineral composition, humus content and particle surface area. Mineral and organic compounds have the ability to bind PrP^{CWD} impacting infectious properties. The extreme variability of these soil constituents suggests that the PrP^{CWD} fate and behavior will depend on specific soil properties. The soil moisture regime also has the potential to affect transportation of compounds through a soil profile. PrP^{CWD} can be bound to soil particles with

3 hypothetical scenarios for prion fate: (i) prions stay in the surface soil horizon and remain bioavailable for grazing animals; (ii) prions can be transported into lower soil horizons and become unavailable for consumption; or (iii) prions can migrate through the soil profile and end up in ground water. We performed bench-scale experiments with soil columns to evaluate the potential for transportation of PrP^{CWD} using soils from different regions of Alberta, Canada. The Luvisols found in northern Alberta have an ustic/udic moisture regime and illite as a predominant clay mineral. The prion binding capacity of illite is poor suggesting it cannot contribute to prion binding and PrP^{CWD} can migrate through the soil profile. Chernozems are found in the CWD-endemic region in southern Alberta and have an aridic soil moisture regime, high amount of humus content and contain montmorillonite. In the Chernozem soil columns PrP^{CWD} remains on the soil surface and does not migrate in lower horizons.

P.67: CWD prion infection of differentiated neurosphere cultures

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Most of the cell culture models of prion infection are based on rodent cell lines found to be permissive to rodent-adapted prions. In order to develop more diverse cell culture systems permissive to infection with naturally occurring prions in humans and animals, one possible strategy is use of neural stem cell cultures from transgenic (Tg) mouse lines expressing prion protein (PrP) of the native host species. Neural stem cells are self-renewing, multipotent progenitors that can be cultured as suspended cell aggregates termed neurospheres. Building on our previous work with mouse-adapted prions, we aimed to establish a cell culture model that would support replication of non-adapted cervid-origin CWD prions by using diffferentiated neurosphere cultures from cervid PrP expressing Tg mice.

Primary neurosphere cultures were isolated from brains of neonatal PrP-null mice and Tg mice expressing cervid PrP-E226 (Tg5037 mice). The neurosphere cultures (NP0 and NP5037, respectively) were expanded by passage >10 times in serum-free media supplemented with N-2 factors, epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) and then differentiated by withdrawal of i) EGF ii) bFGF iii) both bFGF and EGF with adding fetal bovine serum under adherent culture conditions. Upon infection with brain homogenates from CWD positive white-tailed deer or elk, only the EGF-withdrawn cultures (dNP5037/bFGF) accumulated substantial levels of proteinase K-resistant PrP (PrPres). Importatntly, PrPres was not detectable in infected dNP0/bFGF cultures, indicating dNP5037/bFGF cultures supported de novo PrPres formation. The neurosphere cell culture model offers an alternative approach for prion infectivity assays in various species.

P.68: Inter-domain structure in the copper-bound cellular prion protein revealed by site-directed spin labeling and EPR spectroscopy

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The cellular prion protein (PrP^{C}) is a membrane-anchored glycoprotein consisting of 2 domains: a flexible N-terminal domain that participates in metal binding, and a

mainly helical C-terminal domain that converts to β-sheet structure in the course of prion disease. These two domains have traditionally been thought of as non-interacting; however, recent cellular and biophysical evidence has forced a reconsideration of this view. We recently reported a novel tertiary fold in which the Zn^{2+}-bound octarepeat domain contacts the exposed surface of helices 2 and 3.[1] The apparent stability of this interaction was diminished in several mutant PrPs that result in familial prion disease, suggesting a potential role for inter-domain structure in disease progression. In the present work, we examine inter-domain structure in Cu^{2+}-bound recombinant PrP using site-directed spin labeling of the genetically encoded unnatural amino acid pAcPhe and electron paramagnetic resonance (EPR) spectroscopy. Distance measurements between Cu^{2+}, bound with high affinity to the octarepeat domain, and spin labeled residues of the globular C-terminus reveal that the copper-bound octarepeats interact with a negatively charged surface defined by helices 2 and 3. Our results are supported by molecular dynamics simulations and indicate that this *cis* interaction is stabilized by electrostatics. Our findings suggest that metal-induced tertiary structure may be a general property of PrP^{C}, and that disruption of this interaction may be a contributing factor in prion disease pathology.

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P.69: Distinct pathological phenotypes of Creutzfeldt-Jakob disease in recipients of prion-contaminated growth hormone

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The peripheral administration of growth hormone (GH) from prion-contaminated cadaveric pituitary glands is believed to be causative of iatrogenic Creutzfeldt-Jakob disease (iCJD) in more than 225 subjects worldwide. The present study describes the neuropathology and molecular features of 3 of the 30 identified iCJD cases among the approximately 7,700 recipients of cadaveric pituitary hormone in the US National Hormone and Pituitary Program (NHPP). All three cases were methionine (M) homozygous at codon 129 of the prion protein (PrP) gene (GH-CJDMM) and all received NHPP hormone produced before 1977 when a new hormone purification protocol was introduced that reduced the risk of prion contamination. Neuropathological examination revealed divergent phenotypes. The first phenotype, observed in the most recent US NHPP GH-CJD case, was characterized by the presence of amyloid plaques and reminiscent of sCJDMV2-K and, to some extent, variant CJD (vCJD). The second phenotype showed no plaques and shared several, but not all, characteristics with

the sCJDMM(MV)1 subtype. However, PK-resistant PrP^{Sc} ($resPrP^{Sc}$) from GH-CJDMM co-migrated with $resPrP^{Sc}$ type 1 (GH-CJDMM1) of sCJDMM1, but not with type 2 of sCJDMV2-K. Histopathological phenotypes with or without plaques also have been described in 2 groups of Japanese dura mater (d) graft-associated CJD (dCJD) with the same 129MM genotype but apparently different gel mobility of $resPrP^{Sc}$ type 1. Our study suggests that phenotypic diversity in these iatrogenic diseases reflects adaptation of different exogenous prion strains to the 129MM host and/or to different locations of the initial PrP^{C} to PrP^{Sc} conversion.

P.70: Experimental transmission of chronic wasting disease to sheep and goats

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The persistence of chronic wasting disease (CWD) in North American cervids, coupled with efforts to eradicate scrapie in sheep and goats, necessitates an understanding of the transmission, clinical and diagnostic characteristics of CWD in small ruminants. Oral and intracerebral transmission studies were conducted in sheep and goats using tissues from CWD-infected elk. Four lambs and 4 goats were orally inoculated with a pooled brain and lymph node homogenate from a group of farmed elk with clinical CWD. At study endpoint, there was no evidence of primary CWD transmission in the sheep or goat tissues examined by ELISA, western blot and immunohistochemistry (IHC). Two lambs which were challenged intracerebrally with the same pooled elk inoculate displayed neurological signs beginning at 27 months postinoculation (mpi) and were euthanized within 10 d of each other at 28 mpi. Testing of tissues by ELISA and IHC confirmed disease transmission and revealed differences in the distribution and intensity of PrP^{d} deposition between animals. Western immunoblot analysis identified characteristics permitting the differentiation of CWD in sheep from other prion diseases in small ruminants. CWD-infected tissue from the intracerabrally-inoculated sheep has undergone secondary passage into sheep and goats and currently shows no evidence of oral transmission in rectal mucosa biopsies at 20 mpi. These findings corroborate evidence of a significant species barrier preventing the oral transmission of CWD to sheep and goats, and identify diagnostic characteristics to enable the differentiation of prion diseases affecting small ruminants.

P.71: Predicting new prion candidates in yeast

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Prions are infectious proteins capable of self-propagating and transmitting between organisms. Even though there is no homolog to the mammalian prion protein in yeast, several soluble proteins can form heritable aggregates de novo. These proteins provide a model system to investigate the nucleation, aggregation and propagation steps involved in the formation of a prion fibril.

Several prion prediction algorithms have been developed to predict yeast proteins that have the propensity to form prions. One of these algorithms was previously developed in our laboratory (Prion Aggregation Prediction Algorithm, PAPA, Toombs et al., 2012). Therefore, we used PAPA to scan the yeast proteome to identify proteins that contain domains predicted to have prion activity (prion-like domains). These prion-like domains were tested in 4 prion activity assays to assess their activity *in vivo* as well as *in vitro*. Here we provide the preliminary results from our *in vivo* prion activity assays. Using these preliminary results, we are currently investigating a couple

respective full-length proteins for prion activity by developing phenotypic assays.

Ultimately, we may identify new prion candidates in yeast, which will contribute information about the parameters necessary for prion formation and insight into the functions prions play in yeast. In addition, by confirming PAPA's ability to predict prion proteins from the yeast proteome, it allows the possibility to apply this methodology to other proteomes.

P.72: Polymorphisms in prion protein amino acid 170 do not alter the high susceptibility of red-backed voles (Myodes gapperi) to chronic wasting disease

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The β2-α2 loop structure of the cellular prion protein (PrP^C) has been identified as a potential determinant of a host's susceptibility to chronic wasting disease (CWD). The NMR structures of PrP^C from species that possess an asparagine at position 170 (170N; deer, elk, bank voles) show a β2-α2 loop that is more rigid than PrP^C possessing a serine at that same residue (170S; mice, cattle, humans). The 170N genotype is also associated with CWD susceptibility whereas 170S appears to contribute to CWD resistance. We identified and captured a population of red-backed voles that display a natural asparagine/serine polymorphism at position 170. We bred these voles to produce pups with homozygous (170SS, 170NN) or heterozygous (170SN) genotypes and intracerebrally-challenged them with white-tailed deer (*Odocoileus virginanus*) CWD. Following challenge, all 3 genotypes of voles displayed high attack rates of disease (100% for 170NN and 170SN cohorts, and 89% for 170SS cohort) and statistically-indistinguishable survival times (333 ± 28 d post-infection (dpi), 334 ± 53 dpi, and 349 ± 30 dpi, respectively; median survival time ± 95% confidence interval). Clinical signs of disease were similar across experimental challenge groups and included lethargy, ataxia, and decreased burrowing activity. The glycoform profiles and deposition of the abnormal prion protein were assessed by immunoblot and immunohistochemistry analyses. Our work suggests that susceptibility to white-tailed deer CWD in red-backed voles is not reduced by the presence of 170S in the β2-α2 loop of PrP^C.

P.73: Oral challenge of goats with atypical scrapie

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Atypical scrapie transmission has been demonstrated in sheep by intracerebral and oral route (Simmons *et al.*, Andreoletti *et al.*, 2011) but data about goats are not available yet. In 2006 we orally challenged four goats, five months old, with genotype R/H and R/R at codon 154. Animals died starting from 24 to 77 months p.i. without clinical signs. They all resulted negative for scrapie in CNS and peripheral tissues using Western blot and immunohistochemistry. Nevertheless these goats could still represent carriers. This hypothesis was investigated through bioassay in tg338 mice, a sensitive animal model for atypical scrapie infectivity. By end-point dilution titration, the starting inoculum contained $10^{6.8}$ ID50/g. In contrast, all tissues from challenged goats were negative by bioassay.

These negative results could be explained with the low infectivity of the starting inoculum, which could have been unable to induce

disease or infectivity within our period of observation. However the challenge conditions could have been a bias too: as the matter of the fact, while the oral challenge of classical scrapie is still effective in sheep 6–10 months old (Andreoletti *et al.*, 2011), Simmons et al. (2011) demonstrated a very short efficacy period for atypical scrapie (24 hours after birth), hypothesizing that natural transmission could occur mainly via milk. Our work suggests that this could be true also for goats and it should be taken into account in oral challenges. However a low susceptibility of goats to atypical scrapie transmission via oral route cannot be excluded.

P.74: Transmission of experimental CH1641 scrapie to wild-type mice

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Introduction. CH1641 was isolated in the UK in 1970 from a natural case of scrapie in a Cheviot sheep and was further passaged intracerebrally in sheep. CH1641 has been the subject of extensive research because of the biochemical similarities of PrP^{res} from CH1641- and BSE-affected sheep brains. Previous attempts to transmit CH1641 to wild type mice have been unsuccessful. We report here for the first time, the positive transmission of experimental CH1641 to RIII mice and compare the incubation period, PrP^{Sc} profile and PrP^{res} Western blot properties to those of known scrapie and BSE reference strains.

Methods. The CH1641 brain homogenate used in this study came from a pool a 5 sheep brains which had been challenged intracerebrally with brain material from the third passage of CH1641 in sheep. Groups of 15–20 RIII mice were inoculated intracerebrally with a 10% brain homogenate of CH1641. The brains of the mice were examined by PrP^{Sc} profiling and triplex Western blot as reported previously.

Results. Surprisingly CH1641 transmitted to RIII mice with a 100% attack rate although with a long incubation period (794 ± 149 d). The resulting PrP^{Sc} profile was unlike any of the profiles of the scrapie and BSE reference strains reported previously. Triplex Western blot pointed after first passage to a very low PrP^{res} level. We observed a reduction of molecular mass of the non-glycosyl PrP^{res} moiety and concomittant N-terminal 12B2 epitope signal. In comparison to the original CH1641 inoculum there was a lack of a dual population of PrP^{res}.

P.75: Development of pre-mortem diagnosis for suspected Creutzfeldt-Jakob diseases' patients

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Creutzfeldt-Jakob disease (CJD) is the most representative human prion disease caused by abnormal accumulation of misfolding prion protein. The diagnosis is performed with features of magnetic resonance imaging, electroencephalogram and elevated the 14-3-3 protein findings, prion protein gene polymorphisms. In laboratory, the protein detection and analysis of the gene polymorphisms have been monitored, and then clinicians determined as CJD patient or not CJD case combining specific clinical opinions in Korea. We aimed evaluate the epidemiological tendency, and the possibility of early diagnosis through the application of clinical features included the protein tests and genetic analysis. We detected 14-3-3 protein, and analyzed PRNP genotypes for suspected

cases (2010–2014). The results were combined with progressive dementia, myoclonus, and memory decline, and their relationships were analyzed. They were almost within the age range of 60–80 years, and the numbers of male and female were similar. Approximately 49% showed positive for 14-3-3 protein, and the polymorphisms reported to genetic pathogenic factor inherited CJD showed in 11 patients. Three definite and 14 possible sCJD patients defined except for one were positive for 14-3-3, and several probable sporadic cases had pathogenic genetic factors like P102L, E200K and V180I. The clinical presentations showed progressive dementia, visual illusion, myoclonus, ataxia, akinetic mutism, and memory decline. Some MRI and EEG findings showed high signal abnormalities in the fronto-temporal cortex and typical periodic sharp wave complexes. We consider that the active following surveillance for patients would be added to improve the specificity of early CJD diagnosis.

P.76: Deciphering the molecular chaperone network using yeast prions

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Since the discovery in 1994 that known but unexplained non-Mendelian phenotypes in *Saccharomyces cerevisiae* were due to prions, much work has shown the reliance of prion propagation on molecular chaperones. Yeast prions propagate as infectious amyloid fibers composed of misfolded forms of cellular proteins. Replication of these fibers in yeast cells requires new seed generation via breakage mediated by the cellular protein disaggregation system. In recent years we have learned much about the components and mechanisms of various prion-related processes. The various prions rely on the chaperone machinery to different degrees. For example, elevation of the Hsp40 Ydj1 causes curing of the [URE3] prion but has no effect on [PSI^+]. We recently showed elevated Ydj1 reduced availability of a different Hsp40 isoform, Sis1, which is relied upon more by [URE3] than [PSI^+]. Thus, subtle changes in the chaperone landscape lead to measurable changes in the maintenance of prions that may not be observed using alternative assays. In this way using the yeast prion system has produced significant insight into the important functional distinctions that exist between nearly identical chaperone isoforms. These observations have led to new appreciation for the notion that chaperone isoforms that were once thought to be merely redundant in function also perform unique tasks. Here, we highlight several of our recent findings demonstrating the power and utility of the yeast prion system for studying functions, specificity and cooperation of chaperone machinery components.

P.77: Pre-clinical biomarkers of prion infection

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KEYWORDS. Creutzfeldt-Jakob disease, ante-mortem, biomarkers, 14-3-3, neuron-specific enolase

An estimated 1/ 2000 people in the UK are asymptomatic carriers for variant Creutzfeldt-Jakob disease (vCJD), demonstrating a need for screening and early detection. Ante-mortem testing of CJD is currently performed upon clinical presentation of the disease. Surrogate markers are used in combination with other methods for differential diagnosis of CJD; however, existing markers have limitations. The objective of these studies is to define the pre-clinical abundance of known prion disease biomarkers as well as identify novel pre-clinical, ante-mortem markers of prion disease. We

adapted prion disease to rats, facilitating a proteomic approach to a bioavailable fluid (CSF) at preclinical and clinical disease stages. This contrasts with human samples, which are generally only available at clinical stage. The rat CSF proteome was compared between infected rats and age-matched controls through mass spectrometry. A number of proteins up-regulated and/or specific to prion disease were identified. These proteins included CJD biomarkers, 14-3-3s and neuron-specific enolase (NSE), demonstrating the utility of using rat prion disease for pre-clinical biomarker identification. The abundance of these known biomarkers during preclinical stages of the disease will be presented. Since the composition of CSF reflects the pathological processes of the brain, tracking the progression of prion infection in rats will allow us to further define prion disease neurodegeneration.

P.78: Characterization of interactions between the cellular prion protein (PrPC) and toxic assemblies of the amyloid-β peptide

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Alzheimer disease (AD) is a devastating neurodegenerative disease that affects millions of people worldwide, and presents one of our biggest challenges in addressing the health needs of an aging population. Today, multiple lines of research aim to achieve methods for early diagnosis and treatment, as well as a basic understanding of the biology involved in the disease process. Two pathological hallmarks of AD are plaques, comprised primarily of amyloid-β (Aβ) peptide, and neurofibrillary tangles, comprised of the micro-tubule associated protein Tau. Our understanding of how these hallmarks are related to neurodegeneration is still incomplete. However, soluble oligomers of Aβ, which collect prior to or during plaque formation, have been shown to negatively influence cell signaling and synaptic function in vitro. Several receptor targets have been found to link oligomers with cell toxicity, one of which is the cellular prion protein (PrPC). We hypothesize that PrPC can be used to capture and characterize specific toxic oligomers of Aβ, leading to a better understanding of these oligomers and how they interact with PrPC to promote neurodegeneration. Previous studies have implicated mid-size aggregates (10–20mers) or relatively large protofibrils of Aβ as binding partners for PrPC. Our study sheds light on the limitations involved in working with in vitro preparations of soluble Aβ oligomers, and examines more rigorously the characteristics of the PrPC-Aβ complex through the use of multiple surface and solution techniques, including SPR and fluorescence anisotropy.

P.79: Alpha-Synuclein: A potential Cerebrospinal fluid biomarker for differentiation of CJD from AD

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Alpha-Synuclein (Syn-α) is one of the abundant proteins in synapses and its anomalies are associated with various synucleinopathies. Synaptic damage may lead to release of Syn-α in CSF;[1] this release may be prominent in rapid neurodegeneration e.g. CJD and Alzheimer disease through rapid progression (rpAD). Current study involves measurement of CSF Syn-α in sporadic CJD (sCJD) and AD with aim to test CSF Syn-α potential for differential clinical diagnosis. CSF Syn-α in sCJD and AD patients against non-dementia controls were measured using conventional ELISA and an electro-chemiluminescence based system developed using Meso-Scale Discovery^{TM} (MSD) ELISA-plates. Conventional ELISA revealed 2 folds ($P \leq 0.05$) elevated CSF Syn-α in CJD-MM1 (age $= 65.3 \pm 9.1$ year), in comparison to age matched controls (age $= 69.20 \pm 9.5$ year).

Non-significant differences were noticed in VV2 (age = 64.2 ± 10.4 year) in comparison to controls. CSF Syn-α from rpAD cases (age = 65.8 ± 8.6y) were also compared to AD (age = 69.8 ± 8.9 year) and controls (age = 66.6 ± 12.9 year), but no significant differences were noticed. MSD-assay exhibited higher sensitivity than conventional ELISA; in discriminating control and sCJD groups (ROC-AUC of 0.9408 and 0.8435 respectively). CSF Syn-α in sCJD group was noticed to be 6.7 folds higher than control samples ($P \leq 0.0001$). In addition, Syn-α levels were unchanged in AD compared to control cases. In conclusion, our data showed altered level of Syn-α in CSF; and Syn-α can be used as differentiating index for sCJD and AD cases, in contrast to classical AD cases from those with rapid progression course.

KEYWORDS. Alpha-Synuclein, AD, sCJD, Cerebro-spinal fluid, ELISA, Neuro-degeneration

Reference

1 El-Agnaf, et al. FASEB J 2003; PMID:14519670

P.80: Predicting prion propensity in human proteins

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In humans only a single prion-forming protein named PrPc (for "cellular prion protein") is currently known, yet many more neurodegenerative disorders involve aberrant protein aggregation. The classical model for these diseases has involved cell-autonomous aggregation, assuming that aggregation occurs independently in each cell within a diseased patient. However, more recent models have proposed a non-cell-autonomous progression of disease in which aggregates formed in one cell may be transmitted to neighboring cells. These aggregate seeds then cause aggregation of the soluble protein in the "infected" cells, similar to the prion diseases. Within the past few years, a number of proteins that exhibit prion-like aggregation and spread to neighboring tissues have been discovered in patients with Amyotrophic Lateral Sclerosis (ALS). Although ALS has been studied for a number of decades, these proteins were only recently linked to ALS by chance. This demonstrates a clear need for an accurate method to systematically identify additional proteins that may play a pathological role in neurodegenerative disorders. Taking advantage of the compositional similarity of these proteins to the known yeast prions, I plan to use the prion prediction methodology that our lab has pioneered to develop an entirely new algorithm specifically suited for this class of neuronal proteins.

P.81: Optimization of liposomes for in vivo delivery of PrP^C siRNA to the brain

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Prion diseases, or Transmissible Spongiform Encephalopathies (TSEs), primarily affect sheep, cattle, cervids, and humans. The emergence of prion diseases in wildlife populations and the increasing impact of prion diseases on human health has led to an increase in the study of antiprion compounds. Recent studies have found antiprion compounds that can inhibit the infectious prion isomer (PrP^{Res}) or down regulate the normal cellular prion protein (PrP^C). These compounds are often found through the screening of drug or chemical compound libraries. However, most of these chemicals cannot cross the blood brain barrier to effectively inhibit PrP^{Res} formation in brain tissue or to specifically target neuronal PrP^C. Also, these compounds tend to have multiple off target effects, and are often too toxic to use in animal or human subjects. Therefore, we have proposed using intravascular siRNA that is targeted toward PrP^C as a safer and more effective antiprion compound. To protect the siRNA from serum degradation and RES elimination, we have encapsulated it within anionic

PEGylated liposomes using protamine sulfate. Encapsulation of the siRNA with protamine sulfate results in ~90% encapsulation efficiency as compared to 40–80% efficiency without protamine. We are also using the small peptide RVG-9r to target the siRNA to nicotinic acetylcholine receptors within the CNS. In order to reduce the elimination of the peptide, we have covalently bonded it to the PEG groups of the liposomes using carbodiimide reactions. In future experiments, we will determine the effectiveness of this drug delivery system using flow cytometry.

P.82: Investigation of RNA-binding proteins with prion-like domains

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Recently, mutations in a number of RNA-binding proteins have been linked to various neurodegenerative diseases. Many of these proteins are involved in stress granule and processing body (P-body) formation. Stress granule and P-body levels increase when cells are subjected to stress and decrease once the stress is eliminated. Reversible aggregation of these complexes is required for normal cellular function; however, mutations can make some of these proteins more aggregation prone, causing accumulation of protein aggregates. Several of these proteins appear to form these assemblies via regions termed prion-like domains (PrLDs). These domains contain amino acid compositions similar to those of known prions. Our lab is interested in developing the ability to predict prion and aggregation propensities of different proteins in yeast. PAPA (Prion Aggregation Prediction Algorithm) was designed by our lab to predict the prion propensity of Q/N-rich amino acid sequences. Using predictions from this algorithm, we are investigating how mutations made in the PrLDs of different RNA-binding proteins alter stress granule and P-body dynamics in yeast. We are utilizing fluorescence microscopy to monitor the formation of aggregates in vivo and thus observe the effects of different mutations. An understanding of how mutations affect the formation of stress granules and P-bodies in yeast may help to elucidate the mechanisms of similar disease-relevant proteins in the future.

P.83: Gerstmann-Sträussler-Scheinker disease with F198S mutation: Selective propagation of PrPSc and pTau upon inoculation in bank vole

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Gerstmann-Sträussler-Scheinker disease with F198S mutation (GSS-F198S) is characterized by the presence of PrP amyloid plaques as well as neurofibrillary tangles with abnormally-phosphorylated tau protein (pTau) in the brain. The relationship between tau protein and PrP in the pathogenesis of GSS-F198S is unknown. In a previous study, we inoculated intracerebrally 2 GSS-F198S cases in 2 lines of voles carrying either methionine (Bv109M) or isoleucine (Bv109I) at codon 109 of PrP. GSS-F198S transmitted rather efficiently to Bv109I, but not to Bv109M.

Here we investigated the presence of pTau, as assessed by immunohistochemistry with anti-pTau antibodies AT180 and PHF-1, in the same voles previously inoculated with GSS-F198S. Among these voles, most Bv109I showed clinical signs after short survival times (~150 d.p.i.) and were positive for PrPSc. The remaining Bv109I and all Bv109M survived for longer times without showing prion-related pathology or detectable PrPSc. All Bv109I which were previously found PrPSc-positive,

were immunonegative for pTau deposition. In contrast, pTau deposition was detected in 16/20 voles culled without clinical signs after long survival times (225–804 d.p.i.). pTau deposition was characterized by neuropil threads and coiled bodies in the alveus, and was similar in all voles analyzed.

These findings highlight that pTau from GSS-F198S can propagate in voles. Importantly, pTau propagation was independent from PrPSc, as pTau was only found in PrPSc-negative voles surviving longer than 225 d.p.i. Thus, selective transmission of PrPSc and pTau proteinopathies from GSS-F198S can be accomplished by experimental transmission in voles.

P.84: Transmission of chronic wasting disease allotypes into mice expressing elk PRNP

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Chronic Wasting Disease (CWD) is natural prion disease affecting cervids. Polymorphisms in the cervid *PRNP* alleles influence host susceptibility and the properties of CWD agents. This study explores the cross-species transmission of CWD prions derived from white-tailed deer expressing 4 different *PRNP* genotypes, (Wt/Wt, Wt/H95, Wt/S96, H95/S96) into transgenic mice expressing PrP^{C} from Rocky Mountain elk. Upon onset of clinical disease, sagittal sections of brain were taken for histological and biochemical evaluation. As expected, there was no transmission barrier present to TgElk mice inoculated with elk CWD, the mice presented disease earlier than those with the deer isolates, at 105–110 d post inoculation (dpi). TgElk mice infected with the white-tailed deer CWD isolates presented with similar incubation periods, ranging from 101–125 dpi. The exception was the H95/S95 isolate which had a significantly longer incubation period of 140–174 dpi. Brain sections from TgElk infected with elk CWD agent, immunostained with BAR224, revealed intense staining with widespread PrP^{CWD} distribution. The cerebrum, hippocampus and brainstem are all affected by the elk CWD. In contrast, PrPCWD deposition in the TgElk infected with white-tailed deer isolates is less extensive and more regionalized. Further histological, biochemical and bioassay is underway to characterize the CWD agents propagated in TgElk mice.

P.85: Improving Creutzfeldt-Jakob disease incidence estimates by incorporating results of neuropathological analyses, United States, 2003–2011

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Introduction. The incidence of invariably fatal prion diseases such as Creutzfeldt-Jakob disease (CJD) can be estimated by analyzing death certificate data, but there are limitations.

Methods. Prion disease decedents were identified from the US national multiple cause-of-death data and the National Prion Disease Pathology Surveillance Center (NPDPSC) database for 2003–2011. Due to limited personal identifying information, an algorithm was constructed to determine likely decedent matches between the 2 databases. NPDPSC decedents with a positive prion disease autopsy or biopsy result or genetic mutation for whom no match was found in the multiple cause-of-death data were added as cases for incidence calculations; those with negative neuropathology results but

with a death certificate indicating prion disease were removed. The resulting average annual age-adjusted incidence was then calculated.

Results. A total of 2986 decedents were identified as having prion disease indicated as a cause of death in the multiple cause-of-death data; 469 additional NPDPSC decedents were identified with positive neuropathology and/or genetic findings, while 140 decedents with death certificates indicating prion disease had negative neuropathology results. Incorporating the matched data, the average annual age-adjusted incidence of CJD in the United States was 1.2 per million.

Conclusion. Analysis of multiple cause-of-death data is an efficient means of conducting CJD surveillance. However, not all decedents are captured as the death certificate may not list the diagnosis; conversely, a CJD diagnosis on the certificate may be contradicted by neuropathology results. Incorporating findings from NPDPSC neuropathological and genetic analyses produces an estimate closer to the true incidence of the disease.

P.86: Estimating the risk of transmission of BSE and scrapie to ruminants and humans by protein misfolding cyclic amplification

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To assess the risk of the transmission of ruminant prions to ruminants and humans at the molecular level, we investigated the ability of abnormal prion protein (PrP^{Sc}) of typical and atypical BSEs (L-type and H-type) and typical scrapie to convert normal prion protein (PrP^{C}) from bovine, ovine, and human to proteinase K-resistant PrP^{Sc}-like form (PrP^{res}) using serial protein misfolding cyclic amplification (PMCA).

Six rounds of serial PMCA was performed using 10% brain homogenates from transgenic mice expressing bovine, ovine or human PrP^{C} in combination with PrP^{Sc} seed from typical and atypical BSE- or typical scrapie-infected brain homogenates from native host species. In the conventional PMCA, the conversion of PrP^{C} to PrP^{res} was observed only when the species of PrP^{C} source and PrP^{Sc} seed matched. However, in the PMCA with supplements (digitonin, synthetic polyA and heparin), both bovine and ovine PrP^{C} were converted by PrP^{Sc} from all tested prion strains. On the other hand, human PrP^{C} was converted by PrP^{Sc} from typical and H-type BSE in this PMCA condition. Although these results were not compatible with the previous reports describing the lack of transmissibility of H-type BSE to ovine and human transgenic mice, our findings suggest that possible transmission risk of H-type BSE to sheep and human. Bioassay will be required to determine whether the PMCA products are infectious to these animals.

P.87: Mapping N-terminal/C-terminal inter-domain interactions in PrPC through chemical crosslinking and tandem mass spectrometry

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The normal physiological function of PrPC, the cellular form of PrP, and what role it plays in prion diseases remain elusive. Several lines of evidence suggest that the flexible, N-terminal domain (NTD) of PrPC may be responsible for certain toxic activities. For example, we have shown a 21 amino acid deletion within the N-terminal domain induces spontaneous neurodegeneration in transgenic mice and ionic currents in cultured cells, with the polybasic region (residues 23–31) being essential for these effects. Several ligands are known to bind to the NTD, among them the divalent metal ions copper (II) and zinc(II), also suggesting a critical biological function for this region.

NMR studies have previously revealed metal ion-driven structural changes in recombinant PrP. Upon chelation of Cu2+ or Zn2+ by the octarepeat region, the NTD transiently docks with the CTD. Taken together, these observations suggest that intramolecular interactions between the NTD and CTD may play a role in regulating the physiological functions of PrPC. To further investigate this hypothesis, we have employed chemical crosslinking to capture NTD/CTD docked states, followed by analysis by tandem mass spectrometry to identify cross-linked residues. Preliminary evidence suggests that the presence of Cu2+ induces increased cross-linking between residues in the NTD and those in the CTD. Further studies are underway using a variety of constructs and crosslinkers.

P.88: Prion infection interferes with rab7 membrane attachment and lysosomal degradation

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The prion protein PrP^{c} and its pathogenic isoform PrP^{Sc} are found at the plasma membrane and in the endocytic pathway of prion-infected neuronal cells. The presence of protein aggregates attached to membranes by a glycosyl-phosphatidyl-inositol (GPI-) anchor and previous findings that prion-infected neurons harbor elevated cholesterol levels let us hypothesize that endocytic vesicle trafficking is impeded upon prion infection.

To verify this hypothesis we analysed the membrane association of relevant rab proteins in N2a (mouse neuroblastoma) and ScN2a cells, respectively. Rab proteins are small GTPases important for intracellular vesicle movement and targeting. They shuttle between an inactive cytosolic state and an active membrane-bound state. Membrane association is enabled by prenylation of the proteins at the C-terminus. In the cytosol, they interact with rab GDP dissociation inhibitor (rabGDI) which renders them soluble. When we compared the extractability of rab7, 9 and 11 from membrane preparations of N2a and ScN2a cells incubated with recombinantly expressed rabGDI, no difference was found. However, the overall amount of rab7 associated with membranes was significantly reduced in ScN2a. Rab7 is critical for late endosome to lysosome maturation, and as a consequence of reduced active rab7 we observed a prolongated half-life of epidermal growth factor receptor (EGFR) in ScN2a cells. When we overexpressed the protein NPC1 which enables cholesterol efflux from late endosomes, PrP^{Sc} propagation was significantly increased.

Our data demonstrate that prion infected neuronal cells harbor less active, membrane-associated rab7 levels. As a consequence, lysosomal degradation is inhibited which can result in enhanced PrP^{Sc} accumulation.

P.89: Transcriptomic determinants of scrapie prion permissiveness in cultured ovine microglia

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Introduction. In cultured cells, prion permissiveness is highly dependent on the amino acid sequence and host cellular expression of PrP^{C}; however, PrP^{C} expression alone is insufficient. Thus, additional factors must influence susceptibility to prion infection. To identify which cellular factors are associated with permissiveness and resistance to scrapie prions, we compared the transcriptional profiles of a permissive ovine microglia clone to that of a non-permissive ovine microglia clone using RNA-Seq.

Materials and Methods. Microglia clones with differential prion permissiveness were inoculated with either scrapie-positive or scrapie-negative sheep brainstem homogenates. Prion infection was determined by ELISA and immunoblotting. Five passages post-inoculation, the transcriptional profiles of microglia clones were sequenced using Illumina technology. Raw data were mapped against the domestic sheep reference genome prior to comparative transcriptional analysis.

Results. Twenty-two genes were differentially transcribed. In prion-resistant microglia, genes encoding for selenoprotein P, endolysosomal proteases, and proteins involved in extracellular matrix remodeling, and others were significantly up-regulated ($P < 0.05$). Genes encoding for transforming growth factor β-induced, retinoic acid receptor responder 1, and phosphoserine aminotransferase 1 were up-regulated in prion-permissive microglia ($P < 0.05$). Gene Set Enrichment Analyses identified translation and proteolysis as the most affected pathways.

Conclusions. In prion-resistant microglia, selenoprotein P, endolysosomal proteases, and proteins associated with extracellular matrix remodeling may act in synchrony to inhibit prion aggregation and replication. Prion-permissiveness in ovine microglia may be favored by the amyloid aggregating properties of the transforming growth factor β-induced. Functional studies, however, are necessary to test these associations for causality.

P.90: Flow cytometric detection of PrP^{Sc} in neurons from prion-infected mouse brain

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Generation of an abnormal isoform of prion protein (PrP^{Sc}) in neurons plays the central role in a progression of neurodegeneration in prion diseases. However, the mechanism of neurodegeneration has not been fully understood. Detailed analyses of PrP^{Sc}-positive neurons in the brain are required to clarify pathological changes that occur in prion-infected neurons. Here, we report the establishment of a novel method for the detection of PrP^{Sc} in cells by flow cytometry with the specific labeling of PrP^{Sc} using anti-PrP mAb 132. Neuro2a cells were harvested by treatment with collagenase and fixed with paraformaldehyde. After the treatment with GdnSCN, the cells were stained with mAb 132 for PrP^{Sc} and analyzed by flow cytometer. The mean fluorescence intensity of Neuro2a cells persistently infected with 22L prion strain was 4.2-fold higher than that of uninfected cells, indicating that PrP^{Sc}-specific staining with mAb 132 can be applicable to flow cytometric analysis. Next, we attempted to apply this method to neural cells that were dissociated from brains of mice infected with prions. After gating of the cell bodies based on nuclear staining with 7-AAD and forward and side scatter profiles, the double staining of PrP^{Sc} and neuron-specific marker NeuN clearly distinguished NeuN-positive neurons positive for PrP^{Sc} from NeuN-positive neurons negative for PrP^{Sc}. These results indicate that this method is applicable to the separation of PrP^{Sc}-positive neurons from mice brains by fluorescence-activated cell sorting, and thus allows us to perform prion-infected neural cell-type specific analyses using transcriptome or proteome technique.

P.91: Prion-based regulation of the dynamic changes in ribonucleoprotein complexes

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Prions are over-represented among RNA-binding proteins and components of RNP complexes regulating biogenesis, translation, turnover and cellular distribution of mRNAs. We provide 2 new lines of evidence that prion-based complexes are engaged in dynamic rearrangements in a network of RNA-processing complexes allowing for rapid switching between RNA storage and degradation, and between inhibition and activation of protein synthesis. Analysis of prion-like aggregation directed by the Q/N-rich prion domain of yeast Lsm4, a P-body-associated activator of mRNA decapping, revealed that Lsm4 forms heritable aggregates. The aggregation, that is controlled by chaperones and induced by environmental changes, such as temperature drop to 4°C, leads to the increase in the number of P-bodies and their clustering around Lsm4 aggregates, indicative of the directed modulation of mRNA turnover. Analysis of prion properties of yeast Pub1 and its mammalian homolog Tia1 revealed that this protein participates in 2 distinct self-perpetuating structures, both formed through its Q/N-rich prion domain. One is localized to P-bodies and stress granules, consistent with known role of Pub1/Tia1 in stress granule assembly. The other structure is formed co-operatively by Pub1/Tia1 and Sup35/Gspt2, the eRF3 release factor. This heteroprotein prion is normally present in yeast cells, can be visualized as lines forming along tubulin cytoskeleton and drives the assembly of an RNP complex implicated in maintaining the integrity of microtubule cytoskeleton. We hypothesize that the complex directs tubulin synthesis to the sites of microtubule assembly, and that Pub1/Tia1 functionally shuffles between 2 prion-like structures. Support: NIH grant 7R01GM070934–06 (ILD), HHMI (ERK).

P.92: The prion protein in chemosensitive cells of the carotid body in uninfected and infected hamsters

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The carotid bodies (CB) are highly vascularized mammalian sensory organs consisting of uniquely organized clusters of chemosensitive cells that function to monitor blood gasses and adjust respiration accordingly. The chemosensitive cells are synaptically linked to glossopharyngeal nerve terminals that project into the nucleus of the solitary tract in the medulla and to sympathetic preganglionic neurons, both known to be sites of early prion accumulation in experimental prion pathogenesis. We examined the CBs of uninfected golden Syrian hamsters for the presence of the normal isoform of the prion protein (PrP^C), required for prion replication and spread, to establish whether these structures might be involved in prion neuroinvasion following prionemia. We also examined the CBs of clinically-ill hamsters intracerebrally inoculated with either the HY or DY strain of hamster-adapted transmissible mink encephalopathy (TME) to determine if CB cells accumulate the disease associated form of the prion protein (PrP^d) after infection. PrP^C was identified in the chemosensitive cells in CBs of uninfected hamsters, and PrP^d was detected in the chemosensitive cells of animals inoculated intracerebrally with HY TME infected brain homogenate, but not those animals inoculated with DY TME infected brain homogenate. This is the first report demonstrating that the

chemosensitive cells of the CB express PrP^C and that they are a site of disease associated prion accumulation in clinically ill animals. The results of these studies indicate that the CBs are a possible nexus for the centripetal and/or centrifugal spread of prions between blood and the nervous system in infected animals.

P.93: Modeling genetic prion diseases in transgenic mice expressing mutant bank vole PrP

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Unlike other rodents, bank voles exhibit an unprecedented susceptibility to prions from many different species, a phenomenon solely due the sequence of the bank vole prion protein (BVPrP). We recently demonstrated that transgenic (Tg) mice expressing wild-type (wt) BVPrP containing isoleucine at polymorphic codon 109 develop a spontaneous neurodegenerative disorder that exhibits many of the hallmarks of prion disease, including the generation of prion infectivity. To determine if mutations that cause genetic prion disease in humans affect the manifestation of spontaneous disease, we generated Tg mice expressing BVPrP containing either the D178N mutation, which causes fatal familial insomnia; the E200K mutation, which causes familial Creutzfeldt-Jakob disease; or an anchorless PrP mutation similar to those that cause Gerstmann-Sträussler-Scheinker disease. Despite similar levels of BVPrP DNA and mRNA in the brain, BVPrP protein levels were much lower in Tg mice expressing mutant BVPrP than in Tg mice expressing wt BVPrP, suggesting that the mutations destabilize the structure of BVPrP. Remarkably, these physiological or subphysiological concentrations of mutant BVPrP resulted in highly penetrant spontaneous disease, with mean incubation periods ranging from ~120 to ~460 days. The brains of spontaneously ill mice exhibited prominent prion disease-specific neuropathology that was unique to each mutation as well as a highly proteinase K–resistant PrP fragment. Moreover, the spontaneously formed prions were transmissible to Tg mice expressing wt BVPrP or wt mouse PrP. Our results suggest that BVPrP may facilitate the generation of superior mouse models of inherited human prion diseases.

P.94: Increased infectivity of anchorless mouse scrapie prions in transgenic mice overexpressing human prion protein

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Prion protein (PrP) is found in all mammals mostly as a glycoprotein anchored to the plasma membrane by a C-terminal glycophosphatidylinositol (GPI) linkage. Following prion infection, host protease-sensitive prion protein (PrPsen) is converted into an abnormal, disease-associated, protease-resistant form (PrPres). Biochemical characteristics such as the PrP amino acid sequence and post-translational modifications such as glycosylation and GPI anchoring, can affect the transmissibility of prions as well as the biochemical properties of the PrPres generated. Previous in vivo studies have tested the roles of amino acid sequence and glycosylation on cross-species transmission, but the role of GPI anchoring has not been tested. In the current studies we examined the effect of PrPres GPI anchoring using a mouse-human species barrier model. In this model, anchorless 22L mouse scrapie prions were more infectious than anchored 22L mouse scrapie prions when inoculated into tg66 transgenic mice, which expressed wild-type anchored

human PrP at 8–16 fold above normal. Thus the lack of the GPI anchor on PrPres appeared to reduce the effect of the mouse-human PrP species barrier. In contrast, neither form of 22L prions induced disease when tested in a second transgenic mouse which expressed human PrP at 2–4 fold above normal suggesting that PrP expression level also had an impact on our model.

P.95: Glycosylation of PrP^C is a key factor in determining TSE transmission between species

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The risk of transmission of transmissible spongiform encephalopathies (TSE) between different species has been notoriously unpredictable because the mechanisms of transmission are not fully understood. A transmission barrier between species often prevents infection of a new host with a TSE agent. Nonetheless, some TSE agents are able to cross this barrier and infect new species with devastating consequences. The host PrP^C misfolds during disease pathogenesis and has a major role in controlling the transmission of agents between species, but sequence compatibility between host and agent PrP^C does not fully explain host susceptibility. PrP^C is post-translationally modified by the addition of glycan moieties which have an important role in the infectious process. Here we show in vivo that glycosylation of the host PrP^C has a significant impact on the transmission of TSE between different host species.

We infected mice in which the first (N180T), second (N196T) or both (N180T and N196T) N-glycan attachment sites are disrupted with 2 human agents (sCJDMM2 and vCJD) and one hamster strain (263K). The absence of glycosylation at both or the first PrP^C glycosylation site in the host results in almost complete resistance to disease. Absence of the second glycosylation site has a dramatic effect on the barrier to transmission between host species, facilitating the transmission of sCJDMM2 to a host normally resistant to this agent. These results demonstrate that glycosylation of host PrP^C can dramatically alter cross species transmission and is a key factor in determining the transmission efficiency of TSEs between different species.

P.96: Variable relative contribution of methionine and valine at residue 129 to protease resistant prion protein in heterozygous cases of Creutzfeldt-Jakob disease

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Sporadic Creutzfeldt-Jakob disease (sCJD) is thought to originate from the spontaneous misfolding of the endogenous host prion protein (PrPC) into its infectious prion isoform, PrPSc. By contrast, iatrogenic CJD (iCJD) is associated with exposure to an exogenous source of PrPSc. sCJD and iCJD occur as 3 possible PRNP codon 129 genotypes: patients homozygous for methionine (M129) or valine (V129) and those who are heterozygous at this locus. In CJD patients heterozygous at residue 129, the relative contribution of each allotype to PrPSc is unknown and its influence on prion pathogenesis is poorly understood. We have used mass spectrometry to determine the relative abundance of M129 and V129 in PrPSc from heterozygous cases of sCJD and iCJD, the latter of which are linked to human growth hormone therapy in the United Kingdom. Our

results show that, while the amount of M129 or V129 in PrPSc is variable in heterozygous sCJD patients, PrPSc with V129 is most abundant in the majority of heterozygous iCJD patients. The relative abundance of M129 or V129 in PrPSc did not correlate with CJD type, age at clinical onset, or disease duration. However, the data are consistent with sCJD PrPSc originating from the stochastic refolding of endogenous PrPC and iCJD originating from a non-stochastic, exogenous source of PrPSc. Thus, the relative abundance of M129 and V129 in PrPSc may be indicative of the PrPSc allotype(s) which best converted PrPC to PrPSc and may provide a means to trace back to the origin of CJD infection.

P.97: Scrapie transmits to white-tailed deer by the oral route and has a molecular profile similar to chronic wasting disease and distinct from the scrapie inoculum

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The purpose of this work was to determine susceptibility of white-tailed deer (WTD) to the agent of sheep scrapie and to compare the resultant PrP^{Sc} to that of the original inoculum and chronic wasting disease (CWD). We inoculated WTD by a natural route of exposure (concurrent oral and intranasal (IN); n = 5) with a US scrapie isolate. All scrapie-inoculated deer had evidence of PrP^{Sc} accumulation. PrP^{Sc} was detected in lymphoid tissues at preclinical time points, and deer necropsied after 28 months post-inoculation had clinical signs, spongiform encephalopathy, and widespread distribution of PrP^{Sc} in neural and lymphoid tissues. Western blotting (WB) revealed PrP^{Sc} with 2 distinct molecular profiles. WB on cerebral cortex had a profile similar to the original scrapie inoculum, whereas WB of brainstem, cerebellum, or lymph nodes revealed PrP^{Sc} with a higher profile resembling CWD. Homogenates with the 2 distinct profiles from WTD with clinical scrapie were further passaged to mice expressing cervid prion protein and intranasally to sheep and WTD. In cervidized mice, the 2 inocula have distinct incubation times. Sheep inoculated intranasally with WTD derived scrapie developed disease, but only after inoculation with the inoculum that had a scrapie-like profile. The WTD study is ongoing, but deer in both inoculation groups are positive for PrP^{Sc} by rectal mucosal biopsy. In summary, this work demonstrates that WTD are susceptible to the agent of scrapie, 2 distinct molecular profiles of PrP^{Sc} are present in the tissues of affected deer, and inoculum of either profile readily passes to deer.

P.98: Super-infection of knock-in mouse models of familial prion diseases reveals differences in selective vulnerability

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Why do neurodegenerative diseases tend to target specific brain regions? To begin to address this phenomenon known as selective vulnerability we combined two powerful tools, an allelic series of *Prnp* knock-in mouse lines with the high precision of mouse adapted scrapie strains. The first mouse line modeled fatal familial insomnia (FFI), and in old age spontaneously developed neuronal loss and reactive gliosis in the thalamus. The second line modeled Creutzfeldt-Jakob disease (CJD), spontaneously developing PrP^{res} aggregates, spongiosis, and reactive gliosis in the hippocampus. In both models the cerebellum was also affected: atrophied in the FFI model and loaded with PrP^{res} aggregates in the CJD model. A third mouse line expressed a mutated N-terminal polybasic region (PBR), but was disease free. We therefore challenged our allelic series with two scrapie strains with similar incubation periods (RML and 22L), one of which was reported to

aggressively target the cerebellum in vivo (22L). Based on incubation periods, the FFI mice were highly resistant to both scrapie strains. In contrast, CJD mice were resistant to 22L but very sensitive to RML. The opposite was true for PBR mice which were highly resistant to RML but much more prone to 22L than FFI and CJD mice. Interestingly, multiple markers of neurodegeneration revealed a divergence of responses to the two strains in various brain regions. In particular, various markers of gliosis appeared in different brain regions depending on the mouse line:scrapie strain combination, suggesting the existence of a diversity of glial phenotypes in these brains.

P.99: Preliminary study of infectivity in peripheral tissues from a transgenic model of spontaneous prion disease

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Transgenic mice over-expressing bank vole prion protein (TgL1), carrying isoleucine at PrP codon 109 (BvPrP109I), were reported to develop a spontaneous neurological disease recapitulating the hallmarks of prion diseases. Furthermore, we detected infectivity in the brain of spontaneously sick TgL1, by bioassay in wild type voles (Bv109I), up to 10^{-8} brain dilution. In this study we investigated the distribution of infectivity in peripheral tissues (skeletal muscles, spleen and blood) from terminally sick TgL1 mice by bioassay in wild type voles Bv109I. All inocula coming from skeletal muscle (n = 3) were infectious, resulting in 80–100% attack rate with mean survival times of 127, 145 and 199 dpi. By comparison with brain end point dilution experiments in the wild type vole model, the infectivity in muscles can be estimated to be between 10^2 and 10^5 fold less than in brains. In contrast, spleen derived inocula (n = 4) didn't induce disease in any wild type vole Bv109I. Whole blood inocula (n = 2) transmitted the disease in only 1/23 animals at 375 dpi.

The absence of infectivity in spleen and the presence of infectivity traces in blood could suggest that the LRS doesn't play a major role in the spontaneous disease in TgL1 mice. In contrast, TgL1 mice produce high levels of infectivity in skeletal muscles other than in CNS. Further investigations might help to understand the kinetics and pathophysiological events underlying accumulation of PrP^{Sc} and prion infectivity in muscle, and which cell type/s and functional structure/s are involved in affected muscles.

P.100: Cyclophilin A deficiency exacerbates RML-induced prion disease

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Prion diseases are typically characterized by deposition of abnormally folded, partially protease-resistant prion protein, which is associated with activated glia and increased release of cytokines. This neuroinflammatory response may play a role in disease pathogenesis. Recent studies indicated that cyclophilin A (CyPA) may be a key mediator of the neuroinflammatory response in prion diseases. It was found that CyPA from scrapie-infected brains induced cytokine release from astroglia and microglia *in vitro*. This effect was reduced by both anti-CyPA antibody and cyclosporine A, a CyPA inhibitor

(Tribouillard-Tanvier et al., 2012). However, the role of CyPA *in vivo* was not studied.

To investigate whether CyPA mediates neuroinflammation and influences prion disease pathogenesis, we inoculated $CyPA^{+/+}$, $CyPA^{+/-}$ and $CyPA^{-/-}$ mice with the RML scrapie strain, and monitored the time to onset of neurological signs of disease and survival. Time to onset of disease in $CyPA^{-/-}$ mice was reduced by 3% and 6% compared to $CyPA^{+/-}$ and $CyPA^{+/+}$ mice, respectively ($p < 0.05$). Time to death was also significantly reduced, with $CyPA^{-/-}$ mice surviving 3.5% and 7% less then $CyPA^{+/-}$and $CyPA^{+/+}$ mice ($p < 0.05$ and $p < 0.001$, respectively). Thus, CyPA deficiency exacerbated RML-induced disease. Analysis of glial activation is in progress to establish the contribution of CyPA-mediated neuroinflammation in disease pathogenesis.

Reference

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P.101: Complement receptors and regulatory proteins directly bind prions and assist in pathogenesis

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Complement, a component of the innate immune system, is crucial for establishing prion disease. The work in this study aimed to biochemically characterize the interactions between Complement and prions, as well as determine the role of a Complement regulatory protein in establishing disease. We employed surface plasmon resonance (SPR) to test interactions between a panel of Complement proteins and enriched Chronic Wasting Disease prion rods. Upon identification of CD21/35 as putative prion receptors by SPR, we injected fluorescent rods into the footpad and performed intravital microscopy of the popliteal lymph to test for co-localization *in vivo*. Our findings suggest Complement Receptors 1/2 (CD21/35) are putative prion receptors because not only did we observe an interaction between CD21/35 and prions via SPR, fluorescent prions co-localized with CD21/35 *in vivo* on B and follicular dendritic cells in the popliteal lymph node within an hour of inoculation. We also determined other Complement proteins, including C3 cleavage products and C1q, bound infectious prions using SPR. Lastly, to determine the role of complement regulatory protein factor H in disease pathogenesis, we tested a gene-dose effect in response to mouse-adapted Scrapie prions. Factor H deficient mice survived longer and accumulated less prions than their hemizygous or wild type littermates. These findings identify CD21/35 as prion receptors and, as such, provide viable targets for therapeutic intervention. Preventing either or both CD21/35 and factor H from interacting with prions could delay or even prevent disease.

P.102: Increased membrane permeability by complement factors affect PrP^{Sc} deposition in neurons

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We previously reported that reaction of complement factors induced translocation of phosphatidylserine on the plasma membrane of scrapie-infected N2a cells (Hasebe et al., Virology, 2012). We assessed here if complement factors induces similar consequences in primary-cultured cortical neurons from mouse fetuses. When neurons were infected with the Chandler and 22L strains, PrP^{Sc} was detected by immunoblotting after 10 days post inoculation (dpi). We used normal mouse serum (NMS) as a source of complement factors. NMS treatment at 17 dpi increased uptake of propidium iodide (PI) in the prion-infected neurons time-dependently. However, PI-uptake in the neurons at 24 dpi was largest at 6 h after

the treatment and reduced thereafter. No morphological change was observed NMS-treated neurons. These results suggest that increased PI uptake by NMS treatment resulted from temporarily increased membrane permeability rather than cell death. Next, we examined if the increased membrane permeability influences deposition of PrP^{Sc} in the neurons. NMS treatment at 20 dpi reduced the amount of PrP^{Sc} in the Chandler-infected neurons at 8 days after treatment. On the other hand, the amount of PrP^{Sc} in the 22L-infected neurons increased by NMS treatment at 12 dpi but not at 20 dpi. The change in the amount of PrP^{Sc} was blocked by pretreatment of NMS with anti-complement antibodies. These results suggest that complement factors increased membrane permeability on prion-infected neurons, but the effects on PrP^{Sc} deposition were different among prion strains.

P.103: A novel insertion mutation in *Prnp* causes Gerstmann-Sträussler-Scheinker Disease in transgenic mice

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Recently, the Canadian CJD Surveillance System discovered a novel 24 base pair insertion mutation of *PRNP* in a Gerstmann-Sträussler-Scheinker Disease patient (Hinnell et al. 2011). This mutation is predicted to extend the length of the hydrophobic domain (HD) by 8 residues which is of great interest in PrP^{C} biology because: **i)** the HD is the most highly conserved segment of the protein, **ii)** it has been implicated in many of the proposed functions of PrP^{C} and **iii)** it is thought to be involved in the early structural rearrangements during the transition between PrP^{C} and PrP^{Sc}. We created transgenic mice expressing this mutated allele within the context of murine PrP and determined that these animals develop a spontaneous neurologic syndrome at ages >300 d. The onset of disease in these mice can be accelerated through intracranial inoculation with brain homogenate from clinically ill animals, demonstrating transmissibility. Histopathological analysis of these mice shows vacuolation and prominent gliosis. Biochemical profiling reveals a 7 kDa PrP fragment following exposure to proteinase K or removal of carbohydrates, a characteristic of GSS prions. Molecular dynamics simulations indicate an increase in the proportion of β-sheet content, which may prove useful in deciphering the early structural changes during the process of prion conversion.

P.104: Neuroprotective role of innate immunity in prion infection

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Prion diseases are characterized by the self-aggregation of abnormally folded prion protein into amyloid. This amyloidosis is associated with synaptic and neuronal loss, vacuolation and neuroinflammation in the central nervous system (CNS). Astrocytes, the most abundant immune competent cells in the CNS,

participate in the local innate immune responses triggered by a variety of insults including amyloidogenic proteins such as prion protein, amyloid-β and α-synuclein. It is not clear whether innate immune molecules and cells have neurotoxic or neuroprotective roles in neurodegenerative diseases. In this study, astrocytes in culture were permissive to prion infection under certain conditions. Pretreatment of primary cerebellar granule neurons and astrocytes culture with lipopolysaccharide (LPS) resulted in a dramatic reduction in prion replication compared to non-treated control as determined by Western blot, immunocytochemistry and animal bioassay. Suppression of LPS-induced immune responses in astrocytes, however, increased prion replication. Astrocytes triggered up-regulation of Toll-like receptors and production of cytokines in response to recombinant prion fibrils. Our results suggest that astrocytes may play a neuroprotective role in the early stage of prion infection.

P.105: RT-QuIC models trans-species prion transmission

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The propensity for trans-species prion transmission is related to the structural characteristics of the enciphering and heterologous PrP, but the exact mechanism remains mostly mysterious. Studies of the effects of primary or tertiary prion protein structures on trans-species prion transmission have relied primarily upon animal bioassays, making the influence of prion protein structure vs. host co-factors (e.g. cellular constituents, trafficking, and innate immune interactions) difficult to dissect. As an alternative strategy, we used real-time quaking-induced conversion (RT-QuIC) to investigate trans-species prion conversion.

To assess trans-species conversion in the RT-QuIC system, we compared chronic wasting disease (CWD) and bovine spongiform encephalopathy (BSE) prions, as well as feline CWD (fCWD) and feline spongiform encephalopathy (FSE). Each prion was seeded into each host recombinant PrP (full-length rPrP of white-tailed deer, bovine or feline). We demonstrated that fCWD is a more efficient seed for feline rPrP than for white-tailed deer rPrP, which suggests adaptation to the new host. Conversely, FSE maintained sufficient BSE characteristics to more efficiently convert bovine rPrP than feline rPrP. Additionally, human rPrP was competent for conversion by CWD and fCWD. This insinuates that, at the level of protein:protein interactions, the barrier preventing transmission of CWD to humans is less robust than previously estimated.

P.106: Recovery of agents with 'slow virus' properties from prion positive sheep with scrapie

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The prion theory of 'proteinaceous infectious particles' was proposed after interpreting data in a manner that excluded pathogens as the cause for scrapie, a naturally transmitted neurodegenerative disease of sheep. Prions were offered as foreign entities, but they are host proteins modified in prion disease. Because host prions, modified as PrP^{res}, resist biocides, cause spongiform encephalopathy *and* convert PrP^{C} into PrP^{res}, they transfer prion disease pathology by injection into brains, even if brain tissues containing PrP^{res} were biocide pretreated. When sheep are diagnosed with scrapie many of their PrP^{C} had been converted into PrP^{res}, the cause of scrapie signs and pathology. What caused this conversion? Because pathogens can modify host proteins as pathogenic mechanisms we attempted to culture putative pathogens from sheep with scrapie while excluding the prion pathogenic mechanism. From four prion positive sheep, agents containing nucleic acids were recovered. These agents

express two distinct modes of function. Within host cells their virus-like expression produces virus like particles comparable to those published (Bignami and Parry, 1971 Science 171: 389–390. Payne and Sibley, 1975 Acta neuropathology 31: 353–361. Manuelidis et al., 2007 PNAS 104: 1965–1970). Their prokaryote mode of gene function seems only to occur outside host cells and outside the CNS. Those products are tough structures likely for genome preservation such as seeding fields. These scrapie agents are proposed as hybrid pathogens and are available from the NIAID Repository for Biosecurity and Emerging Infectious Diseases, deposited by LSU.

P.107: Inhibition of the interaction between amyliod-β and prion protein by computational methods: A strategy against Alzheimer's disease

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The inhibition of the interaction between the prion and amyloid-β is a valuable strategy against Alzheimer's disease. However the compounds that bind the N-term_HuPrP such as the porphyrins have poor blood brain barrier permeability. Compounds similar to the porphyrins, with good blood-brain barrier permeability might be ideal drug candidates against Alzheimer's disease. In order to select the porphyrin-like compounds from the ZINC database, we imputed the porphyrin SMILE or its name, and obtained 36 porphyrin-like molecules. From the 36, we selected 29 of them with lipophilicity coefficients in the range (0, 5), and molecular weight less than 500. However, we tolerated some lipophilicity coefficients than proscribed by the Lipinski's rule of 5 because of the uncertainties in the calculated values. To calculate logP or logS coefficient using the Molinspiration algorithm or the AlOGP2.1, we copied the SMILE or draw the structure of the ligand from the ZINC database, imputed it on the platform, and then click on calculate LogP or LogS coefficient. The calculation is performed in a matter of seconds, and the results displayed. The minimum value for LogP being −0.4 for ZINC59380156 and the maximum is 4.55 for ZINC78206861. The ZINC database uses the fragmental method of Molinspiration in the calculation of the Lipophilicity coefficient wich takes into account correction factors that compensate for intermolecular interractions; consequently we suggest the 29 compounds selected from the ZINC database will be used for molecular docking with N-Term_PrP, in order to select the best binding poses for molecular dynamics simulations.

P.108: Successful oral challenge of adult cattle with classical BSE

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Classical Bovine spongiform encephalopathy (C-type BSE) is a feed- and food-borne fatal neurological disease which can be orally transmitted to cattle and humans. Due to the presence of contaminated milk replacer, it is generally assumed that cattle become infected early in life as calves and then succumb to disease as adults.

Here we challenged three 14 months old cattle per-orally with 100 grams of C-type BSE brain to investigate age-related susceptibility or resistance. During incubation, the animals were sampled monthly for blood and feces and subjected to standardized testing to identify changes related to neurological disease.

At 53 months post exposure, progressive signs of central nervous system disease were observed in these 3 animals, and they were euthanized. Two of the C-BSE animals tested strongly positive using standard BSE rapid tests, however in 1 C-type challenged animal,

PrP^{sc} was not detected using rapid tests for BSE. Subsequent testing resulted in the detection of pathologic lesion in unusual brain location and PrP^{sc} detection by PMCA only.

Our study demonstrates susceptibility of adult cattle to oral transmission of classical BSE. We are further examining explanations for the unusual disease presentation in the third challenged animal.

P.109: Development and characterization of an *ex*-vivo brain slice culture model of chronic wasting disease

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Prion diseases have long incubation times *in vivo*, therefore, modeling the diseases *ex-vivo* will advance the development of rationale-based therapeutic strategies. An organotypic slice culture assay (POSCA) was recently developed for scrapie prions by inoculating mouse cerebellar brain slices with prions. However, efforts to develop a POSCA model for chronic wasting disease (CWD) have failed due to the species barrier between mice and cervids. To overcome this difficulty, we adopted a transgenic cervidized (Tg12) mouse model and successfully developed an *ex-vivo* brain slice culture model for CWD. We incubated 350-μm cerebellar slices from Tg12 mouse pups with CWD brain homogenate and washed them thoroughly. With the genotypes of the pups blinded, the cultures were grown over 42–48 days before being tested for CWD infectivity using the recently developed RT-QuIC assay. Slices from Tg12 cervidized pups with *PrP+/−* genotype tested positive but slices from the Tg12 *PrP−/−* genotype did not. Infectivity was present in 11 out of 11 Tg 12 *PrP+/−*, whereas 0 out of 10 Tg12 *PrP−/−*. We have successfully cultured POSCA brain slices infected with RML scrapie as confirmed by RT-QuIC and PK digestion assays. Biochemical studies revealed degenerative changes and oxidative damage in the prion infected slice cultures. Our results demonstrate that combining the brain slice culture model of prion diseases with the RT-QuIC assay offers a promising platform for studying the mechanisms of prion proteinopathies as well as for screening anti-prion therapeutics. (ISU Presidential Wildlife initiative, ISU-CVM Diagnostic lab and NIH ES19267 and NS074443).

P.110: Prion protein gene sequences analysis in twelve sheep breeds of Pakistan

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Prions are considered the only agents of Transmissible Spongiform Encephalopathies (TSEs) and are harmful pathogens of mammals. These infectious agents of host are made up through aggregation of conformational isomers (PrPSc) and encode glycoprotein (PrPC) of 33–35 kDa. TSEs are fatal group of diseases which are neurodegenerative and include chronic wasting disease in deer and elk, Creutzfeldt-Jakob disease (CJD) and transmissible mink encephalopathy (TME) in humans and scrapie in goats and sheep. The accumulation of abnormal form of the normal protein (PrP) is common in all diseases related TSE. This abnormal form of PrP called PrPSc is resistant to proteolysis as well as infectious. Present study was conducted in order to do sequence analysis of prion protein gene in 12 breeds ($n = 129$) of the sheep from all provinces of Pakistan including Azad Jammu and Kashmir. We

amplified 771 bp of PrP gene in selected 12 sheep breeds followed by sequencing. We identified single nucleotide polymorphisms (SNPs) and some heterozygous sites were detected in aligned sequences using CodonCode Aligner. We compared the results with other sheep breeds of the word and reported mammalian species sequences in GenBank NCBI through UPGMA phylogenetic analysis using MEGA 6.1. This study provided useful PrP gene based information in sheep population across the country.

P.111: W8, a new Sup35 prion strain, transmits distinctive information with a conserved assembly scheme

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Prion strains are different self-propagating conformers of the same infectious protein. Three strains of the [*PSI*] prion, infectious forms of the yeast Sup35 protein, have been previously characterized in our laboratory. Here we report the discovery of a new [*PSI*] strain, named W8. We demonstrate its robust cellular propagation as well as the protein-only transmission. To reveal strain-specific sequence requirement, mutations that interfered with the propagation of W8 were identified by consecutive substitution of residues 5–55 of Sup35 by proline and insertion of glycine at alternate sites in this segment. Interestingly, propagating W8 with single mutations at residues 5–7 and around residue 43 caused the strain to transmute. In contrast to the assertion that [*PSI*] existed as a dynamic cloud of variants, no random drift in transmission characteristics was detected in mitotically propagated W8 populations. Electron diffraction and mass-per-length measurements indicate that, similar to the 3 previously characterized strains, W8 fibers are composed of about 1 prion molecule per 4.7-Å cross-β repeat period. Thus differently folded single Sup35 molecules, not dimeric and trimeric assemblies, form the basic repeating units to build the 4 [*PSI*] strains.

P.112: Using proteinase K to study the structure of prions

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Introduction. The secondary structure of prions is composed almost entirely of β-sheet secondary structure. Structural constraints suggest that the β-sheet secondary structure is arranged in a β-solenoid. The β-sheet secondary structure is thought to be responsible for the remarkable resistance prions have to proteinase K (PK) digestion. A detailed analysis of the PK digestion products can be used to study the structure of prions by identifying the location of PK cleavage sites in wild type and GPI-anchorless prions.

Materials and Methods. Mass spectrometry and antibodies were used to identify PK cleavage sites in PrP^{Sc}. The PK cleavage sites (from 23–160) in the wild type 263K and drowsy strains of hamster adapted scrapie were identified. In addition murine-adapted GPI-anchorless prions were digested with PK and analyzed by mass spectrometry to determine the PK cleavage sites throughout the entire protein. Antibody-based analysis was performed by other researchers to determine the PK cleavage sites in human and hamster prions.

Results and Conclusions. The results of these diverse analyses were used to identify the regions of the prion that were accessible to PK digestion. These regions were presumed to be accessible to PK due to flexible stretches connecting the β-strand components in PrP^{Sc}. These data, combined with physical constraints imposed by spectroscopic results, were used to propose a qualitative model for the structure of PrP^{Sc}. Assuming that PrP^{Sc} is a four rung β-solenoid, we have threaded the PrP sequence to satisfy the PK proteolysis data and other experimental constraints.

P.113: The chemistry of prions: Small molecules, protein conformers and mass spectrometry

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Introduction. Prions propagate by converting a normal cellular isoform (PrP^{C}) into the prion isoform (PrP^{Sc}) in a template-driven process. The lysines in PrP^{C} are highly conserved and strongly influence prion propagation, based on studies using natural polymorphisms of PrP^{C} and transgenic animals expressing natural and unnatural PrP^{C} polymorphisms.

Materials and Methods. Prions from different hamster-adapted prion strains were reacted with small molecule reagents. The extent of this reaction was quantitated by mass spectrometry or Western blot-based analysis. Some samples were analyzed to quantitate the loss of infectivity associated with the corresponding lysine acetylation.

Results and Conclusions. The reactivity of each of the prion strains with a given reagent was different. The strains showed differences in the reactivity of the N-terminal lysines, the C-terminal lysine and some of the lysines in the two large α-helicies. Western blotting showed differences in the lysine that was part of the epitope of the mAb 3F4. In addition, a relationship between the extent of the reaction of lysines and the loss of infectivity was observed. The approach of using differences in chemical reactivity can be used to understand the role of other amino acids in prion replication. In addition this approach can be used to understand the role that lysines play in the propagation of other prion-like protein misfolding diseases such as AD, ALS, and PD.

P.114: Using patient-specific fibroblasts and iPSC-derived neurons to uncover cellular phenotypes associated with prion diseases

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Cell biology of prion formation and spread remains incompletely understood, largely because of lack of authentic cell models. We report isolation of fibroblasts from skin tissues, derivation of induced pluripotent stem cells (iPSCs), differentiation of iPSCs into mature neurons, and detection of disease-related phenotypes in fibroblasts and iPSC-derived neurons. Fibroblasts were isolated from skin samples of 23 subjects including 9 asymptomatic subjects carrying 6 different PrP mutations, 4 patients with sporadic CJD (sCJD), and 10 normal controls. Surprisingly, not only protease-resistant PrP was detected with Western blotting but also seeding activity was detected with RT-QuIC in fibroblasts of some PrP mutation carriers or sCJD patients. After iPSCs were derived from fibroblasts of mutation carriers (E200K or D178N) and normal controls, functional mature

iPSC-derived neurons were further differentiated, as evidenced by immunostaining with the neuronal marker Map2 and by patch-clamp recording of GABA-induced current. While migration and glycosylation of PrP from fibroblasts were different from those of brain PrP, iPSC-derived neurons exhibited the PrP profile similar to the brain PrP. Notably, shortened neurites and neuritic-beading, characteristics of neurodegeneration, were more readily observed in iPSC-derived E200K neurons or in iPSC-derived neurons challenged with sCJD brain homogenates compared to neurons derived from iPSCs of normal subjects. Our study has generated patient-specific fibroblasts and iPSC-derived neurons that exhibit cellular phenotypes and seem to be authentic cell models for probing human prion diseases. [Supported by the CJD Foundation Award, NIHNS087588, NIHNS062787, and bridge funding from University Hospitals Case Medical Center.]

P.115: Prion protein protects against renal ischemia/reperfusion injury

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The cellular prion protein (PrP^C), a protein most noted for its link to prion diseases, has been found to play a protective role in ischemic brain injury. To investigate the role of PrP^C in the kidney, an organ highly prone to ischemia/reperfusion (IR) injury, we examined wild-type (WT) and PrP^C knockout (KO) mice that were subjected to 30-min of ischemia followed by 1, 2, or 3 d of reperfusion. Renal dysfunction and structural damage was more severe in KO than in WT mice. While PrP was undetectable in KO kidneys, Western blotting revealed an increase in PrP in IR-injured WT kidneys compared to sham-treated kidneys. Compared to WT, KO mice kidneys exhibited increases in oxidative stress markers heme oxygenase-1, nitrotyrosine, and N^{ε}-(carboxymethyl)lysine, and decreases in mitochondrial complexes I and III. Notably, phosphorylated extracellular signal-regulated kinase (pERK) 1/2 staining was predominantly observed in tubular cells from KO mice following 2 d of reperfusion, a time at which significant differences in renal dysfunction, histological changes, oxidative stress, and mitochondrial complexes between WT and KO mice were

observed. Our study provides the first evidence that PrP^C may play a protective role in renal IR injury, likely through its effects on mitochondria and ERK signaling pathways. [Supported by the National Institutes of Health (NIH) NS062787, NS087588, the CJD Foundation, and bridge funding from University Hospitals Case Medical Center to W.Q.Z. and a Scientist Development Grant (12SDG12070174) from American Heart Association to H.Z.]

P.116: Prion protein promotes kidney iron uptake via its ferrireductase activity

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Brain iron-dyshomeostasis is an important cause of neurotoxicity in prion disorders, a group of neurodegenerative conditions associated with the conversion of prion protein (PrPC) from its normal conformation to an aggregated, PrP-scrapie (PrPSc) isoform. Alteration of iron homeostasis is believed to result from impaired function of PrPC in neuronal iron uptake via its ferrireductase activity. However, unequivocal evidence supporting the ferrireductase activity of PrPC is lacking. Kidney provides a relevant model for this evaluation because PrPC is expressed in the kidney, and $\sim$370 μg of iron are re-absorbed daily from the glomerular filtrate by kidney proximal tubule cells (PT), requiring ferrireductase activity. Here, we report that PrPC promotes the uptake of transferrin (Tf) and non-Tf-bound-iron (NTBI) by the kidney in vivo, and mainly NTBI by PT cells in vitro. Thus, uptake of 59Fe administered by gastric gavage, intravenously, or intraperitoneally was significantly lower in PrP-knock-out (PrP$-/-$) mouse kidney relative to PrP$+/+$ controls. Selective in vivo radiolabeling of plasma NTBI with 59Fe revealed similar results. Expression of exogenous PrPC in immortalized PT cells showed localization on the plasma membrane and intracellular vesicles, and increased transepithelial transport of 59Fe-NTBI and to a smaller extent 59Fe-Tf from the apical to the basolateral domain. Notably, the ferrireductase-deficient mutant of PrP (PrP 51–89) lacked this activity. Furthermore, excess NTBI and hemin caused aggregation of PrPC to a detergent-insoluble form, limiting iron uptake. Together, these observations suggest that PrPC promotes retrieval of iron from the glomerular filtrate via its ferrireductase activity, and modulates kidney iron metabolism.

P.117: The multivesicular body is the major internal site of prion conversion

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Conversion of the properly folded prion protein (PrPc) to its insoluble misfolded amyloid form, PrPsc, is thought to be the crucial event in the pathogenesis of transmissible spongiform encephalopathy. Although there is a consensus that this process occurs along the endocytic pathway, evidence as to its exact localization has so far remained inconclusive. To determine which specific endosomal organelle is the major internal site of prion conversion, we combined cell imaging with biochemical techniques. Treating two chronically prion-infected cell lines, SMB and ScN2a, with calpain inhibitors is known to decrease PrPsc levels, but the mechanism of this PrPsc clearance is incompletely understood. We found that, before being cleared from the cells, PrPsc localized to enlarged multivesicular bodies (MVBs). Interestingly, the same decrease in PrPsc levels was observed when MVB maturation was blocked by knocking down Rab7, Tsg101 or Hrs, or when trafficking from the early endosome was inhibited by overexpressing Rab5 or Rab22. Conversely, PrPsc levels actually increased when traffic out of the MVB was blocked by disabling the retromer complex through a knock down of Vps26 or SNX2. Crucially, enhanced degradation or perturbations in intracellular cholesterol were eliminated as processes contributing to PrPsc clearance,

indicating that the reduction in PrPsc levels was due to its decreased conversion from PrPc, thus demonstrating that the major internal site of prion conversion is the MVB.

P.118: The view from above: The potential of aerial surveillance in quantifying CWD infection rates at the herd level

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Background. Large mammals such as domestic cattle and red deer have been reported to align themselves with the magnetic North Pole. Since chronic wasting disease (CWD) affects the behavior of infected cervids, it may be possible to estimate the infection rate, at a herd level, by determining the changes in the alignment of the animals with respect to the magnetic pole. Using available aerial surveillance data from California, a CWD-free state, the alignment of a free ranging elk was determined.

Materials and Methods. Tomales Point is a 2,600 acre fenced-in preserve inside the Point Reyes National seashore. It consists of open grassland and coastal scrub where native California tule elk (*Cervus canadensis nannodes*) roam freely. These elk were introduced to the preserve in 1978 and are part of a program to rebuild the tule elk population in California. There are no other large mammals inside the fence. The aerial surveillance data was examined and approximately 146 elk were identified and their orientation determined.

Results and Conclusions. Analysis of the alignment data demonstrated that tule elk show no particular alignment in any direction. Further research will need to be performed to determine if CWD infected animals have a greater tendency to align themselves with the magnetic pole. As the cost of high-resolution images decreases and aerial surveillance data becomes more available, it may be possible to use other parameters, such as fawn count per doe to estimate CWD prevalence in an infected herd.

P.119: $PrP^{0\backslash 0}$ mice show behavioral abnormalities that suggest PrP^{C} has a role in maintaining the cytoskeleton

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Background. PrP^{C} is highly conserved among mammals, but its natural function is unclear. *Prnp* ablated mice ($PrP^{0/0}$) appear to develop normally and are able to reproduce. These observations seem to indicate that the gene is not essential for viability, in spite of it being highly conserved.

Materials and Methods. A variety of models were used to better understand the physiological role of PrP^{C}. Wild type (WT) and $PrP^{0/0}$ mice were subjected to a series of standard behavioral tests to detect phenotypic differences. Tissues from these mice were studied at the physiological level. Biochemical differences in mouse derived tissues were examined by mass spectrometry.

Results and Conclusions. $PrP^{0/0}$, but not WT, mice showed a substantially reduced ability to build nests. Age-dependent behavioral deficits in memory performance, associative learning, and basal anxiety were also observed in $PrP^{0/0}$ mice. WT, but not $PrP^{0/0}$, mice showed increases in four neurofilament (NF) proteins levels as they aged. Five other proteins were found to be differentially abundant in older (18 month) WT but not in $PrP^{0/0}$ mice. NF-H phosphorylation was reduced in both $PrP^{0/0}$ mouse and cell models. PrP^{C} ablation was associated with the expression of Fyn and phospho-Fyn, a potential regulator for NF phosphorylation. The number of β-tubulin III-

positive neurons in the hippocampus was diminished in $PrP^{0/0}$ mice relative to WT mice. These data indicate that PrP^{C} plays an important role in cytoskeletal organization, brain function, and age-related neuroprotection. Our work represents the first direct biochemical link between these proteins and the observed behavioral phenotypes.

P.120: A protein polymerization cascade mediates the toxicity of seeded amyloids of non-pathogenic huntingtin in yeast

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A distinct group of human neurodegenerative amyloidoses, including Huntington disease, is caused by expansion of polyglutamine (polyQ) stretches in several otherwise unrelated proteins. Studies in yeast, using the first exon of the human huntingtin (Htt)-encoding gene, demonstrate that the deleterious effect of Htt also correlates with the length of its polyQ. While an N-terminal fragment of mutant Htt with a stretch of 103 glutamine residues (Htt103Q) aggregates and causes toxicity, its wild type variant with a sequence of 25 glutamines (Htt25Q) is not toxic and does not aggregate. Here, we observed that non-toxic polymers of proteins with long polyQ or polyQ interspersed with other residues (polyQX) can seed polymerization of Htt25Q, which causes toxicity. We further showed that toxicity of Htt25Q is related to the ability of its polymers to seed polymerization of the essential glutamine/asparagine (Q/N)-rich Sup35 protein which results not only in depletion of its soluble form but also in sequestration of its essential partner protein, Sup45, through its binding to Sup35 polymers. Prion amyloids of the Q/N-rich Rnq1 protein can also seed Htt25Q polymerization which is accompanied with appearance of Sup35 polymers and growth inhibition. Importantly, just polymers of Htt25Q, but not of Rnq1 or polyQ/QX, seed Sup35 polymerization, suggesting that Htt25Q polymers act as intermediators in seeding Sup35 polymerization. The obtained data provide a novel insight into interactions between amyloidogenic proteins and pathophysiological interrelations between various polyQ disorders.

P.121: Improving therapeutic efficacy of 2-aminothiazoles in PrP prion disease

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The lack of therapeutics that halt or slow neurodegenerative diseases is a growing challenge for societies with aging populations. We recently demonstrated that compounds with the 2-aminothiazole (2-AMT) scaffold could extend survival in PrP prion-infected mice. One of these compounds, IND24, was effective against the RML strain of mouse-passaged sheep scrapie, and chronic wasting disease prions, but did not extend survival in Tg (HuPrP) mice infected with Creutzfeldt-Jakob disease prions.

Upon studying the brains of RML-infected mice treated with IND24, we discovered that a new prion strain emerged. Brain homogenates prepared from RML-infected, IND24 treated mice were used to infect CAD5 cells. These prions were found to be resistant to IND24 at concentrations up to 20 μM. In attempts to avoid the development of drug resistance, we investigated the effect of varying the dose and duration of IND24 treatment of the RML-infected mice. When treatment was started prophylactically, IND24 resulted in a four-fold extension in survival over vehicle-treated controls.

In parallel, medicinal chemistry optimization the 2-AMT scaffold, supported by pharmacokinetic analysis in mice, generated compounds with increased efficacy in cells that reached high concentrations in the brain. Long-term efficacy studies showed up to a three-fold

extension in survival when dosing was started after inoculation. In addition, hits from a second chemical scaffold, the biaryl amides, were also optimized and subjected to efficacy studies. Neuropathological investigations suggest that new strains also emerged from other 2-aminothiazoles. Whether some of these compounds might act synergistically remains to be determined.

P.122: α-synuclein amyloid interaction with prion protein: A putative overlap of 2 neurodegenerative diseases

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While the function of the cellular form of the prion protein (PrP^C) is still under debate, there are several reports implying PrP^C being involved in binding and modulation of the toxicity of amyloids involved in neurodegenerative disorders (such as Aβ oligomers in Alzheimer disease). In this work we investigated whether this is also true for α-synuclein (α-syn), a protein involved in neurodegeneration in Parkinson disease (PD). In this study, we used the same methodology employed to obtain synthetic mammalian prions, to form recombinant mouse α-syn amyloids. We characterized various preparations of α-syn amyloids (using atomic force microscopy and biochemical approaches) and subsequently explored the uptake of these preparations in neuroblastoma N2a cells which express PrP^C (N2aWT) and N2a cells knocked out for PrP^C protein (N2aKO). Our results show that the uptake of α-syn amyloids is lower in N2aKO if compared to control cells. Confocal microscopy and co-localization with sub-compartmental markers revealed that the α-syn amyloids co-internalized with PrP^C, accumulated and trafficked to lysosomes. Furthermore, serial passages of N2aWT cells treated with α-syn amyloids led to sustained accumulation of both, α-syn and PrP. Further work was required to validate the importance of this interaction in disease progression *in vivo*. Thus, we performed stereotaxic injections in *substantia nigra pars compacta* of α-syn amyloids in FVB PrPWT and FVB PrPKO mice. Our findings suggest a role for PrP^C in regulating of α-syn uptake, thus, evidencing a link between the two neurodegeneration associated proteins. This study suggests an overlap between prion disease and PD.

P.123: Substitution of histidine by tyrosine at non-OR copper-binding site promotes prion conversion

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Prion protein is a copper binding protein via histidine residues in the octapeptide repeats (OR) and in the non-OR regions. These structures are located in the disordered N-terminal tail of the protein while no copper binding has been reported in the C-terminal domain. Although the functional implication of copper binding to PrP is not completely clear, it is believed that copper plays an important role in prion diseases. To determine whether copper coordination modulates prion formation, we created several mutant mouse (Mo) PrP constructs by replacing histidine residues at the OR and non-OR regions by tyrosine. These constructs were transfected into ScN2a cells and the efficiency of prion conversion was measured. The results showed that replacing histidines by tyrosines at non-OR region led to increase of PrP^{Sc} levels, while histidine replacement in the OR did not alter prion conversion in cells. In addition, cuprizone removal of copper did not alter the level of PrPSc in MoPrP H95Y containing cells whereas it

increased in wild-type (wt) MoPrP. To test these mutants in vitro, we produced recombinant proteins and carried out a fibrilization assay and compared the lag phases duration among different constructs. The results clearly show that the constructs with tyrosine (H95Y, H110Y, or H95,110Y) required shorter lag phases to aggregate compared to wt MoPrP. Based on these data, we could conclude that substitution of histidine by tyrosine residues at non-OR region can enhance PrP^{C}-PrP^{Sc} conversion process, and that the non-OR copper-binding site may possess a critical role in this process.

P.124: Distinct disease phenotypes produced by a *de novo* generated synthetic prion strain: Conformational instability before adaptation

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Prions are infectious proteins that possess multiple self-propagating structures, which define different strains. The structural information for strain diversity is contained in the folding of the pathological isoform, PrP^{Sc}. Following an *in vitro* protocol, recombinant mouse PrP (recMoPrP) was converted to ultrastructurally different amyloid fibrils without any seeding factor. One of these preparations (recMoPrP#4) efficiently propagated in PMCA using the brains of mice overexpressing PrP^{C} (Tga20) as substrate. RecMoPrP#4 was able to infect either GT1 or N2a cell lines causing the conversion of endogenous PrP^{C} to PK-resistant forms. We next assessed the ability of recMoPrP#4 to propagate *in vivo* after intracerebral inoculation in CD1 mice. The animals did not show any evident prion-like pathology and were culled at the end of their lifespan. The brain of these mice was either used for (i) a second passage transmission or (ii) analyzed by PMCA. The latter revealed the presence of PK-resistant PrP with an uncommon biochemical profile when compared to that of known murine prion strains. This amplified isolate was intracerebrally injected in CD1 mice, which developed disease after a relative short incubation time (~160 days). Immunohistochemical and biochemical analysis revealed the presence of three different PK-resistant prion isolates able to produce a subset of completely different pathologies. The biochemical profiles of the isolates that accumulated in the CNS of these animals were distinct from that of the original amyloid used as inoculum. These results indicate that synthetic prions can assume multiple intermediate conformations before adapting and converging to stable strains.

P.125: Distinct strains of Aβ prions implicated in rapidly progressive Alzheimer disease

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Because over 75% of phenotypic variance of late onset Alzheimer disease (AD) remains unexplained by currently identified risk genes, we aimed to investigate the prion paradigm of AD, specifically the role of structure of the brain amyloid β (Aβ) in remarkably variable rates of clinical decline. Using a tandem of novel biophysical methods, we inventoried and analyzed conformational structural characteristics of Aβ in the cortex of 48 cases of sporadic AD with distinctly different disease durations, and correlated the data with clinical profiles

and genetics. In both hippocampus and posterior cingulate cortex we identified an extensive array of distinct Aβ42 particles that differ in conformational structure, size, and display of N-terminal and C-terminal domains. In contrast, Aβ40 present at low levels did not form a major particle with discernible size, and both N-terminal and C- terminal domains were largely exposed. Rapidly progressive cases are associated with a low frequency of *APOE* e4 allele and demonstrate considerably expanded conformational heterogeneity of Aβ42, with higher levels of distinctly structured Aβ42 particles composed of 30–100 monomers, and fewer particles composed of $<$30 monomers. Our data indicate that Aβ42 generates in the AD brain a broad spectrum of different conformational structures - strains - that may have a potentially different toxicity and accumulation rate. The link between rapid clinical decline and levels of Aβ42 with distinct structural characteristics argue for prion-like mechanism encoding variable propagation tempo and phenotypic characteristics of the disease in distinct structures of Aβ42.

P.126: Ultrastructural and biochemical analyses of prion protein amyloids and the role of lysine and proline residues in amyloid formation

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The pathogenic form of prion protein, PrP^{Sc}, is the infectious agent responsible for transmissible spongiform encephalopathies (TSEs). Efforts to understand and treat prion diseases have been hindered by the absence of a definitive PrP^{Sc} structure. We have recently detailed a parallel in register intermolecular β sheet (PIRIBS) model in which PrP^{Sc} is organized as a β-sheet arranged in a series of tandem hairpins. Stacked assembly of these hairpins gives rise to a prion fibril. Ultrastructural analyses on prion amyloids using electron microscopy revealed fibrils resembling a celery stalk, or half-pipe, with fibril widths consistent with the PIRIBS architecture. Further consideration of the PIRIBS model helped identify a potential obstacle for fibril assembly, namely, electrostatic repulsion between 4 cationic lysine residues contained within a central lysine cluster (CLC) of residues 101–110. Also contained with this region are proline residues 102 and 105 involved in familial Gerstmann-Straussler-Scheinker syndrome. Systematic evaluation of the CLC lysine and proline residues indicated individual P102 or P105 substitution allowed PrP amyloid formation with different PK-resistant amyloid cores, implicating the proline residues in influencing local structure. Combined substitution of P102 or P105 residues with charge neutralization of the CLC lysines by asparagine substitution resulted in very rapid, template-independent amyloid formation with PK-resistant amyloid cores more reminiscent of infectious PrP^{Sc}. Thus, the CLC proline and lysine residues influence the amyloidogenicity of PrP, implicating the CLC as a multifaceted modulator of PrP conversion. Together, these studies provide new ways of conceptualizing the self-propagating, pathogenic structure of infectious TSE prion amyloids.

P.127: Caregiver burden in sporadic Creutzfeldt-Jakob Disease

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Introduction. Caregiver burden is a well-recognized component of dementia care and is widely studied in other neurodegenerative conditions. To our knowledge, no data exist regarding caregiver burden in prion disease.

Method. We conducted a retrospective study examining caregiver burden in subjects of the Johns Hopkins Young-Onset and Frontotemporal Dementias Clinic (2004–2010). We used the Zarit Burden Inventory (ZBI) as the outcome variable for measuring overall caregiver burden and collected data on likely contributory variables including cognitive impairment, functional impairment, neurological impairment, and neuropsychiatric symptoms.

Results. We were able to collate data from subjects diagnosed with Alzheimer disease (AD, $n = 21$), behavioral variant frontotemporal dementia (bvFTD, $n = 33$), language variant frontotemporal dementia (lvFTD, $n = 15$), and sporadic Creutzfeldt-Jakob disease (sCJD, $n = 7$). Caregivers for subjects with bvFTD and sCJD exhibited the highest ZBI scores ($p = 0.026$), Neuropsychiatric Inventory-Q (NPI-Q) total severity scores ($p = 0.004$), and NPI-Q caregiver distress scores ($p = 0.002$). In the final regression model, differences in ZBI scores among diagnostic groups were not affected by cognitive, functional, or neurological impairments. There was an interaction effect between diagnostic category and NPI-Q total severity scores in regard to differences in ZBI score, suggesting that much of the differences in ZBI scores were mediated by neuropsychiatric symptoms.

Conclusion. Caregiver burden is exceptionally high in sCJD, approaching levels observed in bvFTD. Much of differences observed in ZBI scores are likely mediated by neuropsychiatric symptoms. Future studies are needed to study caregiver burden in prion disease and it should be considered a secondary outcome measure in future treatment studies.

P.128: Bioassay using ovine and cervid PrP transgenic mice for discrimination of scrapie and CWD origins in sheep and goats

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As the United States works toward the eradication of scrapie, identifying TSE reservoirs that could lead to disease re-emergence is imperative. Development of transgenic mice expressing either the ovine or cervid prion protein has aided characterization of scrapie and CWD, respectively. We hypothesize that transgenic mouse models will discern whether new incidents of scrapie in sheep and goats with clinical disease originated from CWD exposure. Two transgenic mouse lines (Tg338 and TgElk; minimum 5 mice/strain) were inoculated with brain homogenate from clinically affected animals including sheep or goats with naturally acquired classical scrapie, white-tailed deer with naturally acquired CWD (WTD-CWD), or sheep experimentally inoculated with elk-CWD ($sheep^{elk\text{-}CWD}$). Transmission was assessed via survival analysis and western blot characterization of brain PrP^{res}. WTD-CWD transmitted efficiently to TgElk with all mice culled due to clinical disease, whereas all Tg338 remained asymptomatic at endpoint with no PrP^{res} detected in the brain. Ovine and caprine scrapie transmitted poorly to TgElk with all mice asymptomatic at endpoint and 6.8% brain-positive for PrP^{res}, whereas all Tg338 were culled due to clinical disease. $Shee p^{elk\text{-}CWD}$ yielded Tg338 that were all

asymptomatic at endpoint and were all brain-positive for PrP^{res}. However, $sheep^{elk\text{-}CWD}$ yielded TgElk with 5/22 displaying clinical disease near endpoint but 16/22 brain-positive for PrP^{res}. Furthermore, TgElk-PrP^{res} molecular mass appeared lower when inoculated with caprine scrapie versus WTD-CWD and both molecular masses were yielded when inoculated with $sheep^{elk\text{-}CWD}$. These findings suggest primary passage in Tg338 and TgElk could discern whether scrapie in sheep and goats originated from CWD exposure.

P.129: Elucidating the role of matrix metalloproteases in prion protein proteolysis

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Like many proteins, the cellular prion protein, PrPC, undergoes regulated and constitutive endoproteolysis. These post-translational events include well described alpha- and beta-cleavage, newly described gamma-cleavage, and protease and phospholipase mediated PrPC shedding. The exact purpose of PrPC endoproteolysis is not determined, with increasing evidence for different biological roles of the various N- and C-terminal fragments produced, as well as the full-length protein.

The major PrPC proteolytic cleavage event is alpha-cleavage, and often there is higher expression of the C-terminal cleavage fragment, C1, than full-length PrPC. The exact identity of the protease responsible for alpha-cleavage is contentious, with strong evidence both for and against the involvement of the ADAMs (a disintegrin and metalloprotease) family of proteases. Importantly, this alpha-cleavage event has been linked to prion diseases.

Using various in vitro assays, we have identified the matrix metalloprotease (MMP) family of proteases as key regulators of PrPC endoproteolysis, particularly at the alpha-cleavage site. We have determined MMP2 to be an "alpha-PrPase", producing C1 from full-length human PrP in a recombinant protein assay. Surprisingly, treating cultured cells with a pan-MMP inhibitor (prinomastat, selective for MMPs-2, 3, 9, 13 and 14) increased cell associated C1 levels, highlighting the complex and dichotomous nature of the MMPs in the regulation of alpha-cleavage. However, consistent with this C1 increase, we observed significantly decreased PrPSc levels in prinomastat treated prion infected cells. Our findings provide new insight into the normal biology of PrPC proteolytic processing, as well as a potential new avenue for therapeutic interventions in prion disease.

P.130: PK-induced disassembly of partially unfolded PrP^{Sc} fibers supports a multi-rung architecture of PrP^{Sc} subunits

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Evidence based on solid state NMR strongly suggests that recombinant PrP (rPrP) amyloid fibers are an in-register stack of single-rung "flat" monomers, whose β-strand rich cores span from position ~160 to the C-terminus. In contrast, evidence from, 2D electron crystalography, X-ray fiber diffraction, and cryo-electron microscopy and helical reconstruction suggests that PrP^{Sc} consists of stacks of 4-rung β-solenoids. However, the recently proposed PIRIBS model argues that PrP^{Sc} monomers are

also flat, with rPrP amyloid-like β-strand rich cores extending up to position ~90.

Besides a proteinase K (PK) resistant core spanning ~90–230, PrP^{Sc} has an inner "super-resistant" ~152–230 core that resists partial, reversible unfolding induced by guanidine. We therefore reasoned that if PrP^{Sc} is a multi-rung solenoid, PK-treatment of partially unfolded PrP^{Sc} fibers should necessarily result in their complete disassembly, as the N-terminal "base" of each monomer desintegrates. In contrast, if PrP^{Sc} monomers are flat, fibers would persist after such treatment, as seen when rPrP fibers are treated with PK under conditions that preserve their C-terminal β-strand rich cores, which remain stacked.

Our results show virtually complete disassembly of partially unfolded PrP^{Sc} fibers after PK treatment, supporting a multi-rung, rather than flat, architecture of PrP^{Sc} monomers. As a corollary, PrP^{Sc} fibers would be made up of 2 protofilaments, as has been the general consensus based on the general appearance of negative stain TEM images. In this respect, we present evidence suggesting that fiber segments with a "half-pipe" morphology present in PrP^{Sc} preparations, suggestive of wide, single-protofilament fibers, might be tubulin impurities.

P.131: Sinergistic effects of 2 anti-prion compounds targeting the cellular prion protein

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Mounting evidence suggests that PrP^{Sc} could be a difficult pharmacological target in prion diseases. The discovery of therapeutics capable of reducing PrP^{Sc} aggregates and halt neurodegeneration has so far been unsuccessful. The structure of PrP^{Sc} is poorly defined, and likely to be conformationally heterogeneous, as indicated by the existence of prion strains. The latter represents a relevant problem for drug discovery, as several anti-prion compounds identified so far are strain-specific. Moreover, unknown, misfolded PrP intermediates could be responsible for the neurodegenerative process occurring in prion diseases.

We are seeking to develop a pharmacological strategy that may overcome these problems by targeting PrP^{C} instead of PrP^{Sc}. Since they target the native substrate of prion replication reactions, PrP^{C}-directed compounds could be strain-independent, and also prevent the appearance of any neurotoxic PrP species.

Here, we started to explore this strategy by characterizing 2 known PrP^{C}-directed compounds, a cationic tetrapyrrole [Fe(III)-TMPyP] and chlorpromazine hydrocloride (CPH). By employing a variety of biochemical and biophysical techniques, we confirmed that Fe(III)-TMPyP binds to PrP^{C} with sub-micromolar affinity. Conversely, we found that CPH fails to bind PrP^{C} at biologically relevant concentrations. Instead, this compound exerts anti-prion effects by inducing the internalization of PrP^{C} from the cell surface. Consistent with their different mode of action, we found that Fe (III)-TMPyP and CPH act synergistically to inhibit the cytotoxic effects of a PrP mutant, and to block prion propagation in cell cultures.

Our data suggest that targeting PrP^{C} with multiple, mechanistically distinct approaches could be an effective strategy against prion diseases.

P.132: Hematological shift but no evidence of immunological impairment in goat kids naturally devoid of the cellular prion protein (PrP^C)

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Despite extensive research, the physiological role of the cellular prion protein (PrP^C) is still unclear. A nonsense mutation at codon 32 in the *PRNP* gene of the Norwegian Dairy Goat breed results in an early termination of PrP^C synthesis and renders animals homozygous for the mutation devoid of PrP^C. Hence, these PrP^C free animals, unaffected by genetic confounders, provide a unique model for studying PrP^C physiology and function. Here, we report results of hematological and immunological analyses of goat kids without PrP^C ($PRNP^{Ter/Ter}$), heterozygotes ($PRNP^{+/Ter}$) and normal ($PRNP^{+/+}$) PrP^C expression. We found that $PRNP^{Ter/Ter}$ kids had an elevated number of red blood cells (RBCs), although within reference values. Additionally, RBC volumes were slightly decreased, while neutrophil numbers were increased, strikingly similar to what was described in 10 months old transgenic PrP^C KO cattle. Morphological investigations of blood smears and bone marrow imprints appeared normal. Studies of fundamental immunological parameters such as the relative composition of peripheral blood mononuclear cells (PBMCs) and functional studies of macrophages (phagocytic ability) and T lymphocytes (proliferation after stimulation) demonstrated no significant differences between the *PRNP* genotypes. The cell surface PrP^C levels on PBMCs correlated with the *PRNP* mRNA expression levels and were halved in $PRNP^{+/Ter}$ and absent in $PRNP^{Ter/Ter}$ cells, suggesting that in $PRNP^{Ter/Ter}$ cells, nonsense mediated mRNA decay occurs. In summary, our results indicate a role for PrP^C within the bone marrow, more precisely related to maturation and release of erythroid and granulocytic cells.

P.133: Prion-like characteristics of the bacterial protein Microcin E492

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Microcin E492 (Mcc) is a low molecular weight pore-forming bacteriotoxin. Active Mcc is produced only during the exponential phase of growth, whereas Mcc activity is inhibited at the stationary phase by formation of amyloid-like aggregates in the culture similar to those associated with many human diseases. We show, in a similar manner as prions, Mcc naturally exists as two 2 conformers: a β-sheet-rich, protease-resistant, aggregated, inactive form (Mcc^{ia}), and a soluble, protease-sensitive, active form (Mcc^{a}). Exogenous addition of culture medium containing Mcc^{ia} or purified *in vitro*-generated Mcc^{ia} into the culture of bacteria producing Mcc^{a} induces the rapid and efficient conversion of Mcc^{a} into Mcc^{ia}, which is maintained indefinitely, changing the bacterial phenotype. Mcc^{ia} prion-like activity is conformation-dependent and could be reduced by immunodepleting Mcc^{ia}. Furthermore, using a yeast reporter assay, Mcc was able to confer a heritable change in phenotype when fused to the MC region of Sup35p. Interestingly, an internal region of Mcc shares striking sequence similarity with the central domain of the prion protein that has been shown to be a key to the formation of mammalian prions. A synthetic peptide spanning this sequence forms amyloid-like fibrils *in vitro* and is capable of inducing the conversion of Mcc^{a} into Mcc^{ia} in vivo, suggesting that this region correspond to the prion domain of Mcc. Our findings indicate that Mcc is the first prokaryotic protein with prion properties that harnesses prion-like transmission to regulate protein function, suggesting that

propagation of biological information using prion-based conformational switch is an evolutionary conserved mechanism.

P.134: Discussing worries and concerns about prion disease with family members of patients and unaffected anxious people: From clinical counseling experience in Japan

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From nine years of experience through the activities of the prion disease research group in Japan, we have learned what kinds of prion-disease-related psychosocial issues people have. Although their psychological pain is immensely large due to the unexpected difficult condition, most of the people have ability to psychologically adjust to the circumstances over time, and usually, ordinary psychological counseling theories and techniques are useful. However, we have experienced some psychologically "difficult" cases. Some family members have communication problems with medical professionals, which may be solvable by carefully answering their questions and providing good information. People's psychological distress may take the form of anger toward hospital staff. People who are not motivated to pursue the psychological adjustment process are also difficult, but, education about psychological process may be able to motivate them. Also, when prion disease evokes some familial problems and tension, it may be not easy to be solved. One of the most difficult example is a hypochondriac case. We have experienced those people, who are not affected with prion disease, but are unreasonably worried because they think there is a possibility that they got infected with prion from food, biological drugs, etc. Information provision and ordinary psychological counseling skills are not very helpful for these people. In this presentation, we would like to review the psychological issues of people who face the prion-disease-related situation, and discuss how we can provide psychosocial support and help, including the way how we deal with "difficult" cases.

P.135: Molecular dynamics simulation studies of novel Q212H, V203G, and N173K mutations in prion diseases

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Prion diseases in humans are grouped based on whether they are sporadic, inherited, or acquired. In inherited prion disease, abnormal prion proteins (PrP) are produced through a genetic mutation of which 40 point mutations have been discovered. Three novel mutations V203G, Q212H and N173K have been reported, but remained questionable whether the mutations caused the prion disease. In this research, we preformed molecular dynamics simulations and structural analysis to investigate if these novel CJD related mutations behave similarly to known disease causing mutations in the same region of the protein. The results show similar dynamic behavior to pathogenic mutations V203I and Q212P, but differ when compared to the non-pathogenic N171S polymorphism. All three mutations V203G, Q212H and N173K showed a decrease in the protein's overall stability, an slight increase in flexibility, a major loss in salt bridges in the first and second helix, changes in the electrostatic surface of PrP and an increase in the solvent exposure of the protein, all of which are common dynamic behaviors of among pathogenic prion mutations.

P.136: Mother to offspring transmission of CWD—Detection in fawn tissues using the QuIC assay

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To investigate the role mother to offspring transmission plays in chronic wasting disease (CWD), we have employed a small, polyestrous breeding, indoor maintainable cervid model, the Reeves' muntjac deer. Muntjac doe were inoculated with CWD and tested positive by lymphoid biopsy at 4 months post inoculation. From these CWD-infected doe, we obtained 3 viable fawns. These fawns tested IHC-positive for CWD by lymphoid biopsy as early as 40 d post birth, and all have been euthanized due to clinical disease at 31, 34 and 59 months post birth. The QuIC assay demonstrates sensitivity and specificity in the detection of conversion competent prions in peripheral IHC-positive tissues including tonsil, mandibular, partotid, retropharyngeal, and prescapular lymph nodes, adrenal gland, spleen and liver. In summary, using the muntjac deer model, we have demonstrated CWD clinical disease in offspring born to CWD-infected doe and found that the QuIC assay is an effective tool in the detection of prions in peripheral tissues. Our findings demonstrate that transmission of prions from mother to offspring can occur, and may be underestimated for all prion diseases.

P.137: Non-adaptive prion amplification (NAPA): Interspecies prion propagation liberated from the constraints of species-specific PrP primary structure

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Conventional wisdom holds that optimal prion disease progression requires related PrP^{Sc} and PrP^{C} primary (1^0) structures. Accordingly, barriers to transmission of prions from a particular species are generally overcome by expression of PrP from that species in mice. In contrast, interspecies transmissions are commonly characterized by low attack rates/long, variable incubation times on 1^0 transmission, followed by relatively facile transmission on serial passage. Here we explore a distinct variety of host range modifications as prions transit between mice expressing different PrP primary structures which challenge these long held, widely accepted notions. We refer this type of interspecies transmission to as non-adaptive prion amplification (NAPA). In contrast to adaptive prion replication, during NAPA, prions composed of recipient PrP^{Sc} formed after overcoming a species barrier not only fail to further propagate in the recipient species, but remarkably retain the host range properties of prions from the original species, despite significant 1^0 structural differences. Since our findings apply not only to experimentally-adapted prion strains, but also to naturally occurring prions, including transmissible mink encephalopathy and scrapie, they signify greater host range malleability and more frequent asymptomatic prion replication during interspecies transmissions than previously thought. Of particular significance, previous studies describing vCJD/BSE transmissions to mice expressing human or bovine PrP that were hard to understand in the context of adaptive prion replication, are reconciled within the framework of NAPA. Further exploration of this phenomenon

is therefore essential in order to refine future assessments of zoonotic risk, and to fully understand prion transmission mechanisms.

P.138: Coexistence of classical and CH1641-like scrapie prions in experimentally scrapie-affected sheep

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Scrapie is a prion disease in sheep and goats. Abnormal prion protein (PrP^{Sc}) of a few naturally-occurring scrapie cases in sheep is reminiscent of CH1641 scrapie isolate, which is characterized by a lower molecular mass of unglycosylated form of PrP^{Sc} as compared to that of classical scrapie. We have also reported that CH1641-like scrapie prion appears after intraspecies transmission of classical scrapie prion. However, it is still unknown whether strains occur a real mutation process or some strains already existed in the inoculum. In this study, we ask if classical and CH1641-like scrapie prions coexist in sheep. Brains and spleens taken from sheep experimentally affected with classical or CH1641-like scrapie were homogenized and intracerebrally inoculated into wild-type and transgenic mice (TgOvPrP59) overexpressing the ovine prion protein ($A_{136}R_{154}Q_{171}$ sequence). By western blot analysis, CH1641-like specific PrP^{Sc} profiles were confirmed in TgOvPrP59 mice inoculated with sheep brain homogenate affected with CH1641-like scrapie. Wild-type mice inoculated with the same brain homogenate never developed prion disease. Surprisingly, wild-type mice inoculated with sheep spleen homogenate affected with CH1641-like scrapie developed the disease. TgOvPrP59 mice inoculated with the same spleen homogenate developed 2 different forms of scrapie. Diseased TgOvPrP59 mice with a relatively shorter incubation period accumulated CH1641-like specific PrP^{Sc}. While the mice with a longer incubation period accumulated classical scrapie specific PrP^{Sc}. Taken together, these results suggest the coexistence of classical and CH1641-like scrapie prions in spleen of sheep experimentally affected with scrapie.

P.139: Tissue distribution and *in utero* transmission of chronic-wasting disease-associated prions in free-ranging Rocky Mountain elk

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The presence of disease-associated prions in tissues and bodily fluids of chronic wasting disease (CWD)-infected cervid has received much investigation, yet little is known about CWD mother to offspring transmission. Our previous work demonstrated that mother to offspring transmission is efficient in an experimental setting (34). To address the question of relevance in a naturally-exposed free-range population, we have assessed maternal and fetal tissues derived from 19 elk dam-calf pairs harvested from Rocky Mountain National Park (RMNP), a known CWD endemic region. Conventional immunohistochemistry (IHC) identified 3/19 CWD positive dams, whereas a more sensitive assay – the serial protein misfolding cyclic amplification (sPMCA) – detected cervid prions (PrP^{CWD}) in 15/19 dams. PrP^{CWD} tissue distribution, as demonstrated by sPMCA, was widespread and included the central nervous system (CNS), lymphoreticular system (LRS), reproductive, secretory, excretory and adipose tissues. Interestingly, 5 of the 15 sPMCA

positive dams showed no evidence of PrP^{CWD} in either CNS or LRS, sites typically assessed in diagnosing CWD. Analysis of fetal tissues harvested from the 15 sPMCA positive dams revealed PrP^{CWD} in 80% of fetuses (12/15), regardless of gestational stage. These findings demonstrate that PrP^{CWD} is more abundant in free-range elk peripheral tissues than current diagnostic methods suggest, and that transmission of prions from mother to offspring may contribute to the efficient transmission of the CWD in native cervid populations.

P.140: Profiling infectious elk prion protein distribution based on size using asymmetrical flow field-flow fractionation

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The separation of macromolecules in prion-infected biological samples is a challenging task. Size exclusion chromatography (SEC) is not suitable due to irreversible adsorption on the solid supports of the column packing materials. Asymmetrical flow field-flow fractionation (AF-FFF) is a method of choice, which yields size fractionation similar to SEC and avoids adsorption during separation. The technique allows the separation of particles ranging in size from a few nanometers to several microns. The sizes of prion protein oligomers are considered to drive the toxicity within the brain of infect animals.

We injected elk brain infected with chronic wasting disease (CWD) through an AF-FFF connected to a UV-Vis variable wavelength spectrophotometeter, refractive index detector, multi-angle light scattering (MALS) and dynamic light scattering detector connected to the 140° angle of the MALS detector. Alteration of cross-flow and detector flow parameters enabled selective isolation of different particle sizes. Fractions were collected and analyzed for prion protein using dot blot. Corresponding particle size was calculated from cumulant analysis of scattered light intensities. Despite the broad size, shape, and composition of particles present in the whole brain homogenate, particles can be selectively fractionated by AF-FFF. Adjusting cross-flow parameters is an easy way to adjust separation based on specific characteristics of a brain preparation or particle of interest. Our results suggest that the technique is applicable to profile the oligomeric distribution with in brain preparation and could be extended to find out the differences between strains and species affected by prion aggregation.

P.141: The role of neuroprotective chaperones in protein misfolding diseases

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The misfolding, aggregation and accumulation of proteins is known to be the causal event in many neurodegenerative disorders. Under normal conditions a network of chaperones and proteolytic systems fight against the toxic effects of aberrant proteins. Multiple lines of evidence indicate that an imbalance between the production and clearance of proteins initiates protein misfolding, synaptic dysfunction, synaptic loss and neurodegeneration. To begin to address why molecular chaperones do not protect against the cascade of pathogenic events in neurodegeneration, we have evaluated the chaperone network in 5XFAD mice, a severe model of Alzheimer disease as well as

mice lacking the chaperone cysteine string protein (CSPα) a model of fulminant neurodegeneration. 5XFAD mice overexpress mutant human amyloid precursor protein (APP(695)) with the Swedish (K670N, M671L), Florida (I716V), and London (V717I) mutations as well as human presenilin1 (PS1) harbouring, M146L and L286V that cause familial Alzheimer disease rapidly accumulate Aβ42 and have impaired memory (Oakley et al., 2006). CSPα deficient mice are normal at birth, but postnatally develop an impairment of synaptic function in an activity-dependent manner followed by a fulminant form of neurodegeneration, paralysis and early death between postnatal days 40–60 (Fernandez-Chacon et al., 2004). We have found that an overall collapse of the molecular chaperone network does not occur until very late in the sequence of neurodegenerative events while compensatory mechanisms involving upregulation of specific neuroprotective proteins are at play in some neurodegenerative disorders.

P.142: Chronic wasting disease (CWD) transmission into hamsters

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KEYWORDS. chronic wasting disease, interspecies transmission, prion strains

Chronic wasting disease (CWD) is a contagious prion disease of cervids. The continued expansion of the disease in North America is resulting in the increasing number of mammalian species exposed to this infectious agent. As CWD is able to infect multiple cervid species, it is likely that variation of the agent might occur, due to PrP polymorphisms within and between cervid species. Using Syrian Golden hamsters as a model for interspecies transmission, we infected the hamsters with genetically defined CWD isolates from white-tailed deer as well as with hunter-harvested mule deer and white-tailed deer from Saskatchewan. The majority of the CWD isolates resulted in successful transmission to hamsters. Biochemical and neuropathological analyses suggests differences between the CWD isolates.

P.143: In-silico rational design and optimization of small drug leads for inhibition of prion-like propagation of SOD1 misfolding

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the formation of abnormal aggregates of superoxide dismutase 1 (SOD1) enzyme in neuronal cells. Misfolding and aggregation of SOD1 due to mutations, environmental stress, demetalation or overexpression may cause SOD1 to gain toxic properties, which consequently leads to death of motor neurons and death of ALS patients within 2 to 5 y. Currently, there is no cure for ALS and drugs can increase the longevity of ALS patients only by a small fraction. The ultimate goal of our study is to predict molecular structures of misfolded SOD1 and SOD1 aggregates based on structural restrains derived from the experimental data on conformational antibody recognition of neurotoxic SOD1 species [Neil Cashman lab, University of British Columbia]. These structures will be used as targets for therapeutic intervention in ALS. A new platform for the rational drug design enforced by the molecular theory of solvation (aka 3D-RISM-KH), along with the proprietary protocols for optimization of the delivery of drugs to the brain will be used to support development of new conformational antibodies and drugs against ALS. Along

with the potential for selecting new drugs for clinical studies, this will help to better understand the mechanisms of misfolding and oligomerization of SOD1. In addition, we are interested in understanding the prion-like mechanisms involved in ALS progression which can provide the best targets for the blockage of the disease's progression.

P.144: Transmission of CWD to non-human primates: Interim results of a 6 year risk assessment study on the transmissibility to humans

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Rapid spread and high prevalence of CWD in North American captive and free-ranging cervids have raised concerns about a potential risk to human health. Evidence exists that skeletal muscles might harbor significant amount of prion infectivity which is of great importance to consumers of venison, velvet and other cervid products. In order to assess the risk of primary CWD-transmission, cynomolgus macaques *(Macaca fascicularis)* were inoculated with high-titer brain homogenates of CWD-infected white-tailed deer material by intracerebral, intragastric and dermal scarification routes. Another group obtained a total amount of 5 kg CWD-positive muscle homogenate using a repeated low-dose regimen (each received ~125 applications of 40 g muscle homogenate over a 3 y period). Risk of secondary CWD-transmission via blood or blood-derived products is judged by blood transfusion of monkey-adapted CWD to naive recipients. Based on the *in vitro* conversion of recombinant prion protein, a real-time quaking-induced conversion (RT-QuiC) assay was optimized by using lymph node tissues, cerebrospinal fluid samples and brain homogenate derived from BSE-inoculated macaques. Results have shown robust, sensitive, specific detection, high inter- and moderate intra-assay variances in samples derived from BSE-infected macaques. So far, all analyzed samples from CWD-inoculated macaques did not reveal any seeding activity. Future findings of our risk assessment study will greatly contribute to policy decisions including monitoring of human blood products, CWD surveillance and CWD control in captive and free-ranging cervids. Here we will present an update on the current state of the ongoing project.

P.145: Using yeast as a model to understand the mechanisms that underlie protein aggregation, amyloid formation, and prionization

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Current knowledge of prion biology has been greatly enhanced by studies in *Saccharomyces cerevisiae*, which contains several epigenetic elements known as yeast prions. The yeast prion [SWI^+], whose protein determinant is Swi1, a subunit of an evolutionarily conserved ATP-dependent chromatin-remodeling complex SWI/SNF, was discovered in our laboratory. We showed that the first 38 amino acids of Swi1 were able to aggregate, and maintain and propagate [SWI^+]. However, further deletion to the first 32 amino acids resulted in a dramatic reduction in aggregation, indicating that

the minimal prion domain (PrD) of Swi1 lies between residues 32 to 38. Further analysis showed that the first 33 amino acids of Swi1 are able to aggregate, and maintain a prion conformation in the absence of full-length Swi1, suggesting that this region is likely the minimal PrD of Swi1. Using a newly designed reporter system that can faithfully report the prion status of Swi1, we conducted high-throughput screens to identify compounds that can eliminate or inhibit [SWI^+] and have obtained a number of promising anti-[SWI^+] compounds. We are currently elucidating the hit compounds' mechanism of action and investigating their ability to antagonize PrP^{Sc} and inhibit Aβ-induced toxicity in a mammalian cell culture system. These studies will shed light on the mechanisms of protein misfolding, aggregation, and amyloid fiber formation - all of which are relevant to prion diseases and other amyloid-based neurological disorders.

P.146: Studying β-helical PrP^{Sc} constructs *in silico*

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Misfolding and aggregation of prion proteins (PrP) are believed to cause the transmissible spongiform encephalopathies - lethal neurodegenerative diseases in mammals. The 3D structure of the infectious isoform, PrP^{Sc}, and the pathways of conversion remain largely unknown. We report our attempts to build β-rich aggregates from a bovine PrP106 peptide as well as from a full-length bovine PrP construct using a combination of homology and docking modeling. For this purpose we use published threading data, as well as built and aligned several novel threads. For stability evaluations of the modeled systems, multiple μs-long all-atom molecular dynamics (MD) simulations in explicit solvent have been performed using the Gromacs package. The structure evolution, hydrogen bonding, and essential collective dynamics trends have been analyzed from the MD data. It was shown that most of the smallest stable threaded units comprise 2 β-helical rungs, whereas an inclusion of the third rung does not satisfy the threading rules. Modeling of β-helices from the PrP106 peptide resulted in stable N-term-N-term right-handed β-helical (RH) octamer complexes slightly twisted along the fibril axis and less stable N-term-C-term-C-term-N-term RH complexes. Both RH and left-handed (LH) C-term-C-term complexes maintained the aggregated structure, but exhibited a low β content. However, C-terminus based complexes formed more hydrogen bonds than N-terminus based complexes.

P.147: Small molecule inhibitors of mutant PrPC toxicity and PrPsc formation

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Since the function of cellular prion protein (PrPC) is still poorly understood, discovering compounds that target these functions has been difficult, with candidate compounds typically identified based on their ability to disrupt PrPsc infection/proliferation in cell culture. Our objective is to develop compounds capable of disrupting functionally important PrPC-related pathways. Our laboratory has previously reported on a central region deletion of PrPC (ΔCR) whose expression results in several interesting phenotypes, including 1) selected antibiotic hypersensitivity (DBCA, [1,2]), 2) induction of spontaneous ionic currents[3,4], and 3) acute neuronal toxicity in transgenic mice[5]. We undertook a high-throughput screen to identify inhibitors of ΔCR-induced

antibiotic hypersensitivity, and analyzed their modulation of PrPC-related processes. Several classes of small molecule leads were identified, one of which, designated LD49, we report on here. LD49 potently suppress ΔCR-induced antibiotic hypersensitivity in the DBCA, and also inhibits PrPsc production in prion-infected N2a cells. Interestingly, LD49 shows structural similarity to published ligands[6–8] for α-2-delta, an auxiliary subunit of voltage-gated calcium channels, and known PrPC interactor[9], thus raising the possibility that α-2-delta is the target for LD49. However, we have observed only partial correlation between the α-2-delta binding activity of LD49 analogs from the literature and their activity in the DBCA. Efforts are underway to test the role of α-2-delta in the action of LD49, as well as identifying its target using unbiased strategies. We hope to use LD49 and other lead molecules from our screen to elucidate PrPC-related neurotoxic pathways, and to further develop these pharmacophores for therapeutic intervention.

P.148: Key steric zipper segments govern conversion by mouse and elk prions

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The molecular mechanism by which PrP^C is converted to PrP^{Sc} remains poorly understood, yet is clearly influenced by (1) the conformation of the PrP^{Sc} and (2) PrP^C and PrP^{Sc} sequence similarity. Sequence complementarity is also an important determinant of seeding in other amyloid proteins, such as amyloid-β, α-synuclein and tau.

How do specific amino acid side chains influence prion conversion? Eisenberg and colleagues have shown that the atomic-level structure of amyloid fibrils formed by peptides from PrP, amyloid-β, tau and other amyloidogenic proteins consists of a repetitive motif: β-sheets arranged with self-complementary interdigitating amino acid side chains at the interface.

We hypothesize that sequence complementarity within key short segments of PrP impacts prion conversion across many strains and species. In support of this hypothesis, we have found that amino acid substitutions associated with conformational changes within the β2-α2 loop, or with long-distance interactions between the loop and the third helix, do not impact conversion. Instead, seeded prion conversion appears to be controlled by amino acid sequence within key segments of the host PrP^C. Interestingly, PrP^C variants with substitutions in certain segments are efficiently converted by some mouse prion strains but not others, suggesting that the steric zippers involved in prion conversion may vary by PrP^{Sc} conformation.

This work provides important insights into species barriers to prion transmission as well the molecular basis for self-templating amyloid formation.

P.149: Synthetic amyloid fibrils generated from the N-terminal prion protein fragment 23–144 cause transmissible prion disease in mice

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The Y145Stop mutation in *PRNP*, resulting in the C-terminally truncated prion protein

PrP23–144, is associated with a familial, GSS-like prion disease with extensive PrP-amyloid deposits. Even though previous attempt to transmit this human disease to rodents has not been successful, here we report that Tga20 mice inoculated with amyloid fibrils generated from the recombinant mouse PrP23–144 developed clinical symptoms of prion disease with 100% attack rate. The incubation period in the first passage was 254 ± 12 days; it was reduced to ~210 d in subsequent passages. Neuropathological examination showed severe spongiform degeneration and accumulation of PrP plaques, and Western blot analysis revealed the presence of proteinase K (PK)-resistant PrP similar to that observed in classical strains of scrapie (~25–30 kDa; ~19 kDa upon deglycosylation) as well as smaller PK-resistant fragments of 5–6 and 8–9 kDa. The latter fragments appear to be remarkably similar to those observed in GSS phenotypes. moPrP23–144 amyloid fibrils were also infectious to wild-type mice. Furthermore, these fibrils were effective as a seed for the conversion of brain PrP^{C} using serial PMCA protocol *in vitro*, producing a PK-resistant product similar to that generated *in vivo*. The finding that synthetic PrP23–144 fibrils are *bona fide* prions have important implications for understanding mechanistic and structural aspects of prion protein conversion, suggesting that seeded prion protein conversion to PrP^{Sc} state is initiated by element(s) within the ~110–140 segment (which correspond to the b-core of PrP fibrils as defined by solid-state NMR), from which PK-resistant b-structure propagates into the surrounding regions.

P.150: Assessing the pathogenicity of rare *PRNP* variants by comparing case and control allele frequency

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To date, 64 different genetic variants in the *PRNP* gene have been reported to cause prion disease in humans.[1]The majority of these have been observed in only one or a few patients, and pervasive ascertainment bias, low rates of predictive genetic testing and frequent lack of family history[2–5]make it impossible to evaluate the penetrance of the vast majority of variants. Here we systematically assess the disease penetrance of *PRNP* variants by comparing allele frequencies in >13,000 prion disease cases reported to surveillance centers in 8 countries against allele frequencies in >60,000 control individuals sequenced by the Exome Aggregation Consortium and ~500,000 individuals genotyped by 23 and Me. Reportedly pathogenic variants occur in controls ~100 times more frequently than expected under an assumption of complete penetrance, given the known disease incidence. Variants with the strongest prior evidence of pathogenicity are indeed very rare, consistent with high penetrance. Other variants are enriched by ~4-fold to ~250-fold in cases over controls, indicating that these variants dramatically increase prion disease risk but still yield low lifetime penetrance. Still other reportedly pathogenic variants are reasonably common in control populations, and may confer little or no disease risk. Heterozygous nonsense and frameshift variants truncating the prion protein in its N terminus occur in healthy control individuals, in contrast to C-terminal truncating variants found in prion disease patients, indicating that a 50% reduction in gene dosage for *PRNP* is well-tolerated in humans and supporting the safety of therapeutic reduction of prion protein expression.

P.151: Copper complexation of small peptides modeling the fundamental chemistry of copper complexation to the prion protein

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Metal complexation to prion proteins may implicated in the pathway of aggregation of the prion protein as has been reported for the amyloid-β proteins resulting in insoluble plaques that deposit in the brain and lead to neurodegeneration. In this presentation, we describe our use of small peptides to characterize the metal binding sites to and understand the binding of copper to the model peptides and ultimately also the prion protein. The prion proteins bind metals through their amino and carboxyl terminal groups, nitrogen or oxygen in the backbone, and certain side chains with nitrogen, sulfur, oxygen or that are charged. Common spectroscopic techniques can be used to study these metal binding areas including nuclear magnetic resonance (NMR) and mass spectrometry (MS). ^{1}H and ^{13}C NMR spectroscopy were used to monitor metal ion complexation. ^{1}H NMR spectra allows observation of complexation and ^{13}C NMR allows observation of the binding sites by monitoring changes in the intensity of chemical signals. Complexes formed upon addition of copper salts to peptide solutions will also be identified by electrospray ionization MS. Lastly, reverse micelles will be employed along with NMR techniques to characterize the complexes formed in small inhomogeneous environments like those found in the brain between neurons. If one understands how the metal forms complexes with the prion protein one can develop the knowledge to be used to design compounds that inhibit this complexation and begin to probe the role of the metal ion complexation.

P.152: Relationship of PrPSc molecular properties with incubation time in a natural prion disease host: A characterization of 3 isolates of US sheep scrapie

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Determination of aspects of tertiary and quaternary structure of PrPSc associated with differences in disease presentation in the host is a key area of interest in the prion field. Previously, we determined that a US scrapie isolate (136-VDEP) with a short incubation time upon passage in sheep also exhibited low PrPSc stability in guanidine hydrochloride (GdnHCl), as compared to 2 isolates with longer incubation times and higher GdnHCl stability (136-A and 13–7). Here, we utilize this natural host system for a more in-depth examination of PrPSc biochemical properties. First, we recapitulated the incubation time findings from sheep in ovinized (VV136) transgenic mice via intracranial inoculation of 136-VDEP and 136-A. In contrast to published results in rodent strains, lower GdnHCl stability of 13-VDEP from sheep brain did not correlate with decreased stability of aggregates in the presence of SDS and heat. While the aggregate stability assay involves proteinase K (PK) treatment, the lack of correlation cannot be explained by a separate PK-sensitive population of PrPSc, since all 3 isolates exhibit similar PK sensitivity profiles, without a large fraction of PK-sensitive material. However, a time course of GdnHCl treatment suggests the presence of 2 distinct populations of PrPSc in the brain homogenates. Since oligomeric aggregates have been associated with higher infectivity, we examined PrPSc size distributions by sucrose gradient fractionation, but did not observe differences

between isolates. We suggest that in this system, phenotypical differences may be due to tertiary structural, as opposed to quaternary structural, differences.

P.153: An independent and blinded confirmation of real-time quaking-induced conversion (RT-QuIC) analysis of cervid rectal biopsies for detection of chronic wasting disease

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Prion diseases are transmissible spongiform encephalopathies (TSEs) characterized by an always fatal, progressive neuronal degeneration in the brain due to infectious misfolded prion proteins whose prolonged incubation periods often make ante-mortem diagnosis difficult. Chronic wasting disease (CWD) is a TSE affecting both wild and captive populations of mule deer, white-tailed deer, elk and moose. CWD in cervids was first identified in Rocky Mountain States and has recently spread to several other states including Iowa. In this current study, we attempted to independently confirm the results of a Real-Time Quaking-Induced Conversion (RT-QuIC) assay to diagnose CWD using rectal biopsy sections from farmed white-tailed deer. First, we generated recombinant prion protein substrate and then validated the quality of protein for RT-QuIC using a reference prion protein kindly provided by Dr. Caughey's lab. After validating the assay, we blindly evaluated approximately 350 rectal biopsy samples analyzed previously by another institution. All assay plates included positive and negative controls and were analyzed in triplicate. Samples were analyzed using the Biotek Cytation-3 multimode plate reader for 24-hrs duration. Our RT-QuIC assays showed 55% positivity for 356 rectal samples analyzed. Comparison of RT-QuIC results with the immunohistochemical results of obex revealed 93% sensitivity (95% confidence limits: 88.05–95.78%) and 96% specificity (95% CL: 91–99%), confirming that the RT-QuIC assay may be one of the most promising rapid assays for detecting CWD prions. We are currently working on applying the RT-QuIC assay to other test samples (ISU Presidential Wildlife initiative, ISU-CVM Diagnostic lab and ES10586).

P.154: Brain derived lipids inhibit prion amyloid formation *in vitro*

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The normal cellular prion protein (PrP^C) resides in cellular outer membrane lipid rafts and conversion from PrP^C to the pathogenic misfolded isoform is believed to occur at the lipid membrane. *In vitro* assays have demonstrated the intimate association between prion conversion and lipids, specifically phosphatidylethanolamine, which is a critical cofactor in the formation of synthetic infectious prions. In the current work, we demonstrate an opposing property of lipids, the ability to inhibit amyloid formation *in vitro*. The real-time quaking-induced conversion assay (RT-QuIC) was used to investigate whole brain lipid effects on prion amyloid formation. An alcohol based extraction technique was used to remove the lipid content from terminal chronic wasting disease (CWD)-infected white tailed deer brain homogenates. Eliminating lipids increased the sensitivity of RT-QuIC detection of CWD in brain samples one hundred-fold. Addition of brain-derived lipid extracts to CWD prion samples inhibited amyloid formation in a dose-dependent

manner. Brain-derived lipids also inhibited prion amyloid formation in RT-QuIC reaction seeds derived from lymphoid tissues. This is the first demonstration of brain derived lipids directly inhibiting prion amyloid formation *in vitro* and highlights the diverse roles lipids play in the conversion process. Further experiments will identify the individual lipid species or groups of lipids responsible for this inhibitory activity.

P.155: Quantitative real-time analysis of disease specific tau amyloid seeding activity

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A leading hypothesis for the cause of neurodegenerative diseases is the templated misfolding of cellular proteins to an amyloid state. Spongiform encephalopathies were the first diseases discovered to be caused by a misfolded amyloid-rich protein. It is now recognized that the major human neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), and chronic traumatic encephalopathy (CTE), also are associated with amyloid formation. Moreover, AD and PD amyloids have been shown competent to transmit disease in experimental animal models, suggesting shared mechanisms with traditional prion diseases. Sensitive detection of prion disease has been advanced by *in vitro* amplification of low levels of disease specific amyloid seeds, e.g. serial protein misfolding amplification (sPMCA), amyloid seeding (ASA) and real-time quaking induced conversion (RT-QuIC), thereby replicating the disease process *in vitro*. In addition, measurement of the amyloid formation rate can estimate the level of disease-associated seed by using methods analogous to quantitative polymerase chain reaction (qPCR). In the present work, we apply these principles to show that seeding activity of *in vitro* generated amyloid tau and AD brain amyloid tau can be readily detected and quantitated.

P.156: The cellular form of the prion protein PrP^C is processed to varying degrees in different species and *PRNP* genotypes

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Cellular prion protein PrP^C may be described as a proprotein, in which the active peptides are released by proteolytic cleavage at various positions, e.g. α-cleavage (ovine codon 114) or C-terminal cleavage (ovine codon 231). By this shedding process different PrP^C domains will be released, such as PrP25-114 (also called N1), PrP25-231 or retained on the cell surface, such as PrP115-234 (C1), and full length PrP25-234. The *in vivo* function of these PrP^C fragments and the proteases involved are mostly unresolved, but there is no doubt that these fragments can make up significant amounts of total PrP^C levels in mammalian brain tissue. We have previously shown an association between ovine *PRNP* genotypes and the level of cleavage products in brain suggesting a link with scrapie susceptibility. We have analyzed the steady-state levels of full-length PrP^C, C1 and C2 in brain material from a number of rodent, carnivore and ruminant species. We conclude that there are considerable differences in the ratio of PrP^C to C1 and the presence of C2 among mammalian species. For example gray squirrel cortex had 10% C1

of total PrP^Cwhich was the lowest of all samples analyzed, whereas sheep of the ARR/ARR genotype exhibited 5 times more C1, on average 53% of total PrP^C. Increased total PrP^C expression is associated with the relative level of truncated forms. It is likely that these differences in PrP^C processing contribute to the susceptibility and pathogenesis of prion diseases and they may reflect on diverse biological roles in different species.

P.157: Uptake of prions into plants

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Soil may preserve chronic wasting disease (CWD) and scrapie infectivity in the environment, making consumption or inhalation of soil particles a plausible mechanism whereby naïve animals can be exposed to prions. Plants are known to absorb a variety of substances from soil, including whole proteins, yet the potential for plants to take up abnormal prion protein (PrP^{TSE}) and preserve prion infectivity is not known. In this study, we assessed PrP^{TSE} uptake into roots using laser scanning confocal microscopy with fluorescently tagged PrP^{TSE} and we used serial protein misfolding cyclic amplification (sPMCA) and detect and quantify PrP^{TSE} levels in plant aerial tissues. Fluorescence was identified in the root hairs of the model plant *Arabidopsis thaliana*, as well as the crop plants alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*) and tomato (*Solanum lycopersicum*) upon exposure to tagged PrP^{TSE} but not a tagged control preparation. Using sPMCA, we found evidence of PrP^{TSE} in aerial tissues of *A. thaliana*, alfalfa and maize (*Zea mays*) grown in hydroponic cultures in which only roots were exposed to PrP^{TSE}. Levels of PrP^{TSE} in plant aerial tissues ranged from approximately 4×10^{-10} to 1×10^{-9} g $PrP^{TSE} \bullet g^{-1}$ plant dry weight or 2×10^5 to 7×10^6 intracerebral ID_{50} units$\bullet g^{-1}$ plant dry weight. Both stems and leaves of *A. thaliana* grown in culture media containing prions are infectious when intracerebrally-injected into mice. Our results suggest that prions can be taken up by plants and that contaminated plants may represent a previously unrecognized risk of human, domestic species and wildlife exposure to prions.

P.158: Evaluation of prion vaccine administered with vaccine enhancing agent

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Transmissible spongiform encephalopathy (TSE) is a neurodegenerative disorder characterized by pathologic accumulation of a misfolded form of a normal cellular protein in neurons. Emergence of TSEs in wildlife populations and the ability of some TSEs to cross species barriers have prompted concern regarding the lack of treatment options or prevention strategies. Efforts at vaccine development have been hampered by the difficulty of overcoming self-tolerance. Studies in our lab have demonstrated that vaccine induced immunity is often diminished due to the recruitment of anti-inflammatory myeloid cells. We hypothesized that utilizing an effective antigen while inhibiting monocyte migration could elicit a more effective anti-prion response.

The vaccine was formulated using a peptide fragment of the human prion protein (PrP106-126). This peptide spontaneously forms fibrillar aggregates and is thought to mediate the conversion from the normal cellular prion protein (PrPC) to the pathogenic form (PrPSC). To enhance vaccine efficacy, a monocyte migration inhibitor was administered (RS102895). To further target the pathogenic PrPSC, the peptide was reconstituted in an acidic solution and incubated at 37°C to increase fibrillization. Antibody responses were assessed using

ELISA and Western Blot. Vaccinated mice exhibited increased antibody titers in addition to a cell mediated immune response. Mice treated with RS 102895 also exhibited increased concentrations of antibodies against both PrP 106–126 and PrPC.

This vaccination regime shows great promise in eliciting an immune response, thus overcoming self-tolerance. Our results suggest that this strategy could overcome the limitations that have prevented successful development of a prion vaccine.

P.159: Improvements of nasal brushing procedure for Creutzfeldt-Jakob disease diagnosis

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Introduction. We previously identified prion seeding activity in olfactory mucosa (OM) of 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 a sterile disposable Cyto-brush (Kito-Brush, Kaltek) which might provoke a mild discomfort for patients. Therefore, we aimed to use a more gentle tool for OM samplings such as short nylon fiber Flocked swabs (Copan technologies).

Materials and Methods. We collected OM and CSF samples in 43 CJD patients. To ensure efficient OM sample collection, each patient underwent to two OM samplings using flocked swabs one in each nostril and a final with Cyto-brush. OM samples were processed and analyzed by RT-QuIC, as previously described.

Results. Using Cyto-brushes 32 out of 35 OM samples were positive by RT-QuIC analysis, while flocked swabs in 40 out of 43 OM samples. In contrast, CSF samples were positive in 33 out of 43. Two OM samples resulted negative for both Cyto-brush and Flocked swab. Neither OM sampling technique or CSF produced false positives.

Conclusion. This study demonstrates that OM brushing following RT-QuIC ssay is 95% sensitive and 100% specific in CJD diagnosis while CSF resulted 77% sensitive. OM collection using flocked swabs is preferred and provides same sensitivity as cyto-brush. These data recommend four separate samplings possibly from both nostrils to maximize the sensitivity, using three Flocked swabs and lastly a brush.

P.160: Detecting the temporal status of prionemia in transmissible spongiform encephalopathy-infected hosts

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Infectious prions can traverse epithelial barriers and gain access to the circulatory system early in infection. The details of prion entry, temporal status, and persistence in the blood remain unknown. Furthermore, it is unknown if the route of inoculation plays a role in the development of prionemia. We previously demonstrated PrP^C amyloid conversion activity in the blood (prionemia) of deer and hamsters infected with transmissible spongiform encephalopathies (TSEs) using whole blood real-time

quaking-induced conversion (wbRT-QuIC). In this study we analyzed the temporal status of prionemia, spanning 0–100% of the disease course, in hosts exposed to TSEs via blood transfusion or other peripheral means (i.e. oral, aerosol, extranasal, and subcutaneous). Our results demonstrate the presence of PrP^C amyolid conversion activity in the blood of all TSE-inoculated hosts as early as 15 minutes post inoculation likely-representing the point source inoculum–which was cleared from the circulatory system by 72 hours post exposure. De novo host generated hematogenous PrP^C amyloid conversion activity, or prionemia, was identified at 4–5% of the TSE disease course and persisted throughout disease. Our results indicate that hematogenous prions can traverse mucosal surfaces and enter the circulatory system with the same speed and efficiency as those entering the blood directly by blood transfusion, and that an asymptomatic carrier state is established within minutes of TSE exposure.

P.161: Prion soil binding may explain efficient horizontal CWD transmission

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Background. Chronic wasting disease (CWD) is unique due to the facile spread in nature. The interaction of excreted CWD prions and soil is a hypothesized contributor in environmental transmission. The present study examines whether and to what degree CWD prions bind to silty clay loam (SCL) using an adapted version of real-time quaking-induced conversion (RT-QuIC) methodology.

Materials and Methods. Varying amounts (50–3.12 mg) of SCL were incubated with 1 mL-serial dilutions of CWD (+), CWD (−), or no brain homogenate (BH). Samples were centrifuged, washed, diluted 1:10 in 0.1% SDS, and 2.5 uL seeded in RT-QuIC assays employing recombinant Syrian hamster prion PrP substrate. Multiple well replicates of sample and supernatant fractions were assayed for positive seeding activity (recorded as thioflavin T fluorescence emission; 480 nm). Samples were considered positive if they crossed a threshold of 25,000. Reaction rates (RR) were calculated, averaged, and expressed as 1/RR.

Results. Positive seeding activity was detected for most SCL samples incubated with CWD (+) BH dilutions. Higher SCL concentrations (50 mg) produced low fluorescent readings due to optical interference. Lower SCL concentrations (6.25 mg) produced minimal optical interference and removed the vast majority of seeding activity from CWD+ BH in a concentration-dependent manner; determined by seeding activity in residual BH supernatants. Control SCL and supernatants produced minimal false-positive reactions (8 of 240 replicates; 3.3%). We estimated the prion binding capacity of SCL to be 0.16 ng/mg.

Conclusion. Silty clay loam exhibits highly efficient prion binding, inferring a durable environmental reservoir, and an efficient mechanism for indirect horizontal CWD transmission.

P.162: Prion protein cleavage fragments modulate neural stem cell renewal and migration

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Neural stem cells (NSCs) are now recognized to persist in the brain into old age and possibly throughout life. They present a potential new avenue of therapeutic targeting in neurodegenerative diseases and aging. Understanding the factors that influence their growth will be essential for any therapies that

target these cells. The normal function of the prion protein (PrP) has remained elusive. PrP undergoes at least 2 internal cleavage events to produce N1/C1 and N2/C2 fragments. We have proposed that these distinct fragments possess differing properties and physiological function. Our previous studies have shown that the N-terminal cleavage fragment designated N2 reduces the production of intracellular reactive oxygen species (ROS) in response to mild stress. Other research groups have shown protective effects of N1. NSC growth is modulated by intracellular ROS levels and NSCs harvested from mice expressing different levels of PrP show a positive correlation between PrP expression and growth. We hypothesized that the N2 fragment and also the longer N1 fragment might be able to modulate NSC growth through their effects on modulating intracellular ROS. We find that both the N1 and N2 fragments halt cellular growth, migration and maturation. NSCs show reduced intracellular ROS detection following N1 or N2 exposure and appear to have entered into a quiescent state. Inhibition of NADPH oxidase produces a similar phenotype in these cells. Our investigations now focus on the role of N1 and N2 modulation of NADPH oxidase signaling pathways in maintaining stem cell quiescence.

P.163: A practical approach to avoiding iatrogenic CJD from invasive instruments

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Potential Creutzfeldt-Jakob disease instrument-contamination events continue to occur that involve widespread hospital and patient concern. This paper proposes a combination of diagnostic tests and instrument handling procedures that, if routinely applied to patients admitted with symptoms of either dementia or cerebellar disease, should eliminate the risk of iatrogenic instrument infection.

P.164: Blood transmission of prion infectivity in the squirrel monkey: The Baxter study

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Five vCJD disease transmissions and an estimated 1 in 2000 'silent' infections in UK residents emphasize the continued need for information about disease risk in humans. A large study of blood component infectivity in a non-human primate model has now been completed and analyzed. Among 1 GSS, 4 sCJD, and 3 vCJD cases, only GSS leukocytes transmitted disease within a 5–6 year surveillance period. A transmission study in recipients of multiple whole blood transfusions during the incubation and clinical stages of sCJD and vCJD in ic-infected donor animals was uniformly negative. These results, together with other laboratory studies in rodents and non-human primates and epidemiological observations in humans, suggest that blood donations from cases of GSS (and perhaps other familial forms of TSE) carry more risk than from vCJD cases, and that little or no risk is associated with sCJD. The issue of decades-long incubation periods in 'silent' vCJD carriers remains open.

P.165: A prion protein-derived peptide reduces brain damage in an animal model of stroke

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The conformational duality of the prion protein translates into strikingly distinct fates this protein can impart on the living systems. In one case, the outcome is the irrevocable neurodegeneration and fatal disease. In stark contrast, the cellular form of the prion protein (PrP^c) partakes in life-sustaining cellular functions that include neuroprotection, signal transduction, and angiogenesis. Importantly, PrP^c has been shown to be involved in a natural response to vascular injury, with the cell damage and death due to stroke being significantly increased in its absence. In vascular injury, including stroke, one of the key cell death-triggering events is the release of toxic levels of free hemin. A potential solution to this damage is a peptide therapeutic agent that would neutralize hemin, thus reducing deleterious effects of bleeding. Both the hemorrhagic and ischemic stroke (with "microbleeds" inflicting secondary damage) would benefit from this approach. Using biophysical methods, we have identified a peptide derived from the PrP^c N-terminus and tested a hypothesis that this hemin-sequestering fragment could reduce brain damage due to bleeding. We employed a well-established mouse model of intracerebral hemorrhagic stroke (ICH) to test neurological/behavioral deficiencies and brain tissue damage caused by stroke in the presence and absence of the peptide treatment. Our results indicate efficacy of the tested peptide at reducing deleterious effects of stroke *in-vivo*, making it a strong candidate for further development as a novel therapeutic intervention in vascular injury events.